Lactose digestion and maldigestion : measurement and clinical consequences

Koetse, Harmanna Antje

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1. Het lactose splitsende vermogen van de dunne darm kan betrouwbaar worden gemeten met behulp van de ^{13}C-lactose/^{2}H-glucose test. Daarmee wordt het mogelijk om de relatie tussen genotype en fenotype van lactase expressie bij mensen te bestuderen. *(dit proefschrift)*

2. Het optreden van intolerantiesymptomen na consumptie van lactose wordt meer veroorzaakt door verschillen in verwerking van ongesplitst lactose in de dikke darm dan door de lactase activiteit van de dunne arm en de dunne darm passagetijd. Beïnvloeding van de daarvoor verantwoordelijke factoren kan de tolerantie voor lactose bij mensen met een lage dunne darm lactase activiteit doen toenemen. *(dit proefschrift)*

3. Fysieke activiteit verhoogt de betrouwbaarheid van $^{13}CO_2$ ademtesten doordat de snelheidsbepalende stap verschuift van het metabolisme van de opgenomen componenten naar de vertering en absorptie van het test substraat. *(dit proefschrift)*

4. Dieetvoorschriften hebben ingrijpende medische,sociale en financiële gevolgen en moeten daarom gebaseerd worden op betrouwbare gegevens die de indicatie bepalen.

5. De opname indicatie voor een kind met "onzekere" of "gedecompenseerde" ouders neemt af met de toename van ervaring van de dienstdoende arts

6. In de opleiding tot kinderarts dient meer aandacht te worden gegeven aan (kinder)psychiatrische ziektebeelden, psychologische mechanismen en de invloed van gezinsinteracties op het kind.

7. Denktijd kan een effectieve vorm van werktijd zijn, daarom dient over het creëren van denktijd binnen werktijd te worden nagedacht.

8. Wanneer de indicatie voor verdere behandeling van een patiënt door een "vaste kinderarts" wordt gesteld, is die behandelaar zelden degene die deze indicatie heeft geformuleerd. Veelal betreft het dan ook de constatering van een gecompliceerd medisch en/of psychosociaal probleem. Analyse van dit probleem vooraf kan resulteren in een meer adequaat verwijzingspatroon.

9. De oudste man ter wereld (Yukichi Chugani, ex- zijdeworm teler, geboren in 1889 in Kyushu, Japan), gaf in maart 2002 het geheim van zijn gezondheid prijs: drie matige maaltijden en een glas melk per dag. Gezien zijn genetische achtergrond is de tolerantie voor lactose van deze man opmerkelijk.

10. Voor verbetering van de kwaliteit van klinische zorg is intervisie en een intercollegiale kritische houding essentieel. Dit vereist een open werksfeer, waarin kritiek geven en ontvangen als niet bedreigend worden ervaren.

11. Een bril opzetten is alleen zinvol als je iets beter wilt zien. Te weinig mensen beseffen hoe belangrijk het kan zijn te genieten van de ervaring om iets niet te zien! (Els Haeck, juli 2002)

12. "Wat stelt dat nou eigenlijk voor: wetenschap? Stoerdoenerij.....Volgens de wetenschap stijgt de zeespiegel met tien centimeter per eeuw. Ik heb het voor de aardigheid eens nagemeten: ik kwam al op een stijging van anderhalve meter in zes uur!" (Herman Finkers)

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measurement and clinical consequences

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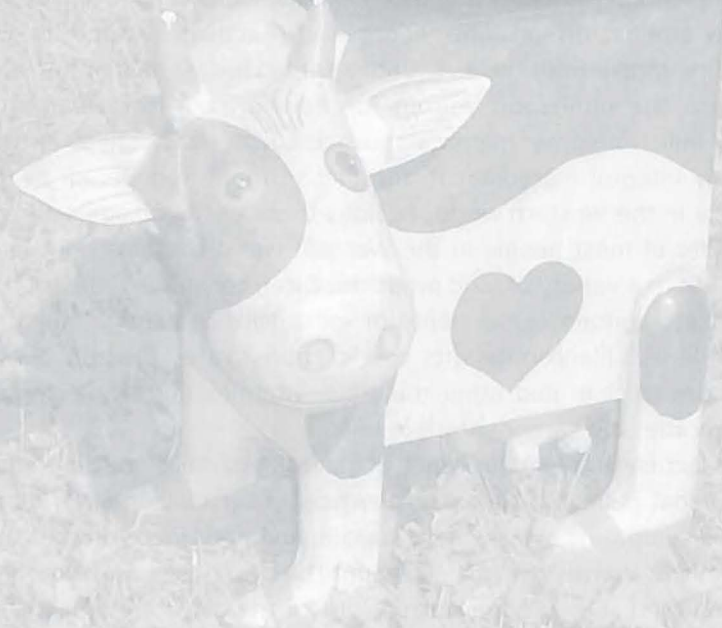
Life is short, science is long; opportunity is elusive, experiment is dangerous, judgement is difficult. It is not enough for the physician to do what is necessary, but the patient and the attendants must do their part as well, and circumstances must be favourable.
(Hippocrates Aphorisms Section I, 4th century B.C)

Contents:

Chapter 1

General Introduction and outline of this Thesis



1.1 INTRODUCTION

Milk and other dairy products are prominent components of the Western diet. They provide high quality proteins as well as vitamins and are the most significant nutritional source of calcium. The ability to digest milk products is an important factor in the prevention of rickets and osteoporosis, especially in climates with a relatively low amount of sunshine, necessary to activate vitamin D, which regulates calcium uptake from the diet. Until weaning breast milk or humanised bottle-feeds are the only food source for newborns. After weaning the consumption of milk from other mammals such as cow's, goats, sheep or camels remains as very integral ingredient in the diet in many regions all over the world, especially in the Western world. Besides these easily recognisable dairy products, the diet of most people in the Western world contains a lot of milk ingredients added to a variety of food products. Often consumers are not aware that food products contain components of milk. Milk contains a mixture of important nutrients. Macronutrients are carbohydrate, protein and fat. Micronutrients are calcium and other minerals, vitamins and trace elements. Lactose is nearly the sole carbohydrate in milk.

Maldigestion of lactose is the major cause of gastrointestinal symptoms such as bloating, abdominal pain, flatulence or diarrhoea after consumption of dairy products. These symptoms are not very specific and may therefore be caused by other mechanisms than lactose maldigestion. The small intestinal brush border enzyme lactase is the only enzyme responsible for the digestion of lactose.

The occurrence of gastrointestinal symptoms after consumption of animal milk has already been recognized by Hippocrates (4th century B.C) as described in his Aphorisms: *"Milk is not recommended for those who suffer from headaches. It is bad too, for patients with fever, those whose bellies are distended and full of rumbling and those who are thirsty. "* (Fragment)[1]. Intolerance symptoms may also be caused by allergic reactions to proteins in mother's milk or animal derived milk products (for instance Cow's milk protein allergy or CMPA), but this will not be further discussed in this thesis.

Since elimination of dairy products from the Western diet has major nutritional consequences for calcium, vitamin and protein intake adequate diagnosis of adverse reactions is necessary. The studies described in this thesis focus on the relationship between lactose (mal) digestion and the occurrence of clinical symptoms. A better understanding of this (possible) cause-effect relation may prevent unnecessarily elimination of milk and milk products from the diet. To study this relation we evaluated the available diagnostic methods of lactose maldigestion and developed new techniques to improve the measurement of human lactose digestion.

1.2 Lactose

1.2.1 *Chemical structure*

Lactose (Galactose ß1,4 Glucose) is a disaccharide consisting of equimolar quantities of two monosaccharides, glucose and galactose.

1.2.2 Biological synthesis

Lactose is synthesised by lactose synthethase in the mammary gland during late pregnancy and lactation and excreted in mammalian milk. Human milk contains 7% lactose. The lactose content of milk is not notably altered by a change in the maternal diet or in the level of blood glucose. The most frequently used animal derived milk in human diets, cow's milk, contains 5% lactose.

1.2.3 Biological functions

Breast milk, which provides approximately 10% of the calories as protein and 45% as fat contains about 45% of the calories as carbohydrate, almost solely lactose. Therefore lactose is an important energy source for newborns. In adults the nutritional contribution of lactose in the diet is less important, since other carbohydrates can also be utilized. For neonates lactose may also have an important function in the bacterial colonisation of the large intestine, since even in neonates not all consumed lactose will be digested under physiological conditions and the spill over from the small intestine will reach the colon.

1.3 Physiology of lactose digestion

Lactose cannot be absorbed by the intact intestinal mucosa. It has to be degraded into its absorbable monosaccharides glucose and galactose.

1.3.1 *Lactase, the enzyme*

Lactase-phlorizine hydrolase (EC 3.2.1.23,EC 3.2.1.62) is the beta-galactosidase enzyme responsible for the hydrolysis of lactose into glucose and galactose. It is synthesised in the small intestinal microvillus membrane.

1.3.2. Physiological regulation of lactase enzyme expression

From the proximal to the distal small intestine mucosa lactase activity is present in a characteristic gradient. Maximal activity occurs in the proximal to mid jejunum and lower activity in the duodenum and ileum[2]. The expression of enzyme activity is high from the 35th gestational week until the weaning period. In the majority of human subjects lactase enzyme activity decreases after weaning to low levels as found in adults, coded for by an autosomal recessive gene[3,4]. The gene is located on chromosome 2[5,6]. However, in a minority of the world population, people originating from the Western world, by genetical determination the capacity to digest lactose after weaning is maintained[7]. Most probably, this is caused by selection of a spontaneous mutation in the lactase-phlorizin hydrolase gene[8]. This mutation is inherited in a dominant autosomal way[9]. With the domestication of cows, goats, camels and sheep, on estimation about 10000 years ago, this mutation was especially of benefit for individuals from populations living in area's with a marginal availability of calcium and vitamin D. Such

circumstances prevailed in the northern parts of Europe. It was hypothesised by Sahi et al[10] that the ability to digest lactose after the weaning period caused a nutritional benefit for individuals with the "lactase persistence mutation". They were able to digest lactose from non-human mammal species, which allowed them to use milk derived from animals in their diet as an easily available source of protein, vitamins, trace elements, calcium and energy. This nutritional advantage caused a longer life expectancy and a higher reproduction rate compared to the individuals without the mutation. Apart from the described protein and energy nutritional benefit of milk consumption, it is presumed that the ability to digest milk could prevent rickets with less pelvic deformations. Therefore women with lactase persistence had a privileged position in the population because of a higher birth rate compared to women without a persistent presence of lactase[11].

1.4 Pathology of lactose digestion

The inability to digest lactose after weaning is the normal condition for most of non-Western individuals in the world population. However before this phenomenon was recognised, these individuals were diagnosed to suffer from "lactase deficiency", which was considered a pathological condition. It is now generally accepted that high lactase activity beyond early childhood is the exception and that low lactase activity after that age is "normal". The prevalence of lactase non-persistency in different populations in the world varies from 4% in Denmark to more than 90% in Asian regions[10].

In infants, children and adults with lactase persistence two other conditions may also cause insufficient lactose digestion.

1.4.1 Mucosal damage of the small intestine

This is the most frequent cause of insufficient lactose hydrolysis in individuals with genetic lactase persistence. Several pathological processes and diseases can cause small intestinal mucosal damage, like celiac disease[12], radiation enteritis[13], cytostatic treatment[14], tropical sprue[15] or Crohn's disease[21,22]. Depending on the localisation and the severity of the mucosal damage expression of all disaccharidases including lactase is impaired.

1.4.2 Congenital absence of lactase enzyme synthesis

Congenital lactase deficiency (CLD) is a very rare autosomal recessive gastrointestinal disorder. In Finland however it is one of the approximately 30 rare recessive disorders that are relatively common. Until 1995 42 Finnish patients have been described, while less than 50 patients have been reported from elsewhere[23]. Affected patients present with watery diarrhoea starting during the first 1-10 days of life when fed lactose-containing milk. The mutation is located on chromosome 2q21 outside the region of the Lactase Phlorizine Hydrolase gene. There is an almost total lack of lactase activity in jejunal biopsies of these patients[23].

1.5 Diagnostic tests used to determine lactose digestion or maldigestion in humans

There are many methods to measure lactose digestion in humans. These methods are based on different principles causing variable accuracy and diagnostic reliability. The possibilities and limitations of the available test methods are discussed below.

1.5.1 Measurement of lactase enzyme activity in a Small Bowel Biopsy Specimen

Lactase activity in a small sample of small bowel mucosa is measured by biochemical methods.

Specimens are obtained through endoscopic biopsy and subsequently homogenised and incubated with lactose. Lactase hydrolyses the substrate into glucose and galactose. The glucose concentration of the supernatant presents the capacity of the enzyme to hydrolyse the substrate. Dahlqvist first described this method in 1968[24]. To correct for confounding influences of water content variations in the specimen, results are expressed as activity units per gram protein. Since only a very small part of the mucosa can be tested, the relation between the measured lactase activity with the over all physiological lactase activity of the small intestine has not been well established. Under circumstances of mucosal damage especially, local variation in degrees of damage can be large (so called "patchy lesions"). Apart from this, the lactose hydrolysis capacity *(in vivo)* is not only dependent on lactase activity but also on the contact time with the substrate.

1.5.2 Lactose Tolerance Test (LTT)

After consumption of lactose the substrate will be hydrolysed into glucose and galactose. After intestinal absorption of both monosaccharides the galactose is converted into glucose in the liver. Depending on the feeding state of the individual, glucose is either stored in the liver or released to the blood. In the fed state most glucose will be stored, while in the fasting state the substrate derived glucose will mainly be released to the blood. The rise in serum concentration of glucose is related to the amount of lactose that is hydrolysed. However, the total glucose concentration in blood partly consists of glucose derived from body stores, which makes the test unreliable. Therefore this test has been abandoned in clinical practice.

1.5.3 *Lactose Tolerance Test with addition of ethanol*

In this test the same substrate and principle as for the LTT is used. The addition of ethanol inhibits the hepatic conversion from galactose into glucose. Therefore galactose will be released by the liver into the blood compartment. Galactose (which has toxic effects) is cleared from the blood by renal excretion. The concentration of galactose in the urine thus represents the hydrolysis of lactose in the intestine and the subsequent uptake of galactose by the intestinal mucosa. Because of the toxic effects of the obligatory ethanol suppletion and the toxic

effects of galactose this test cannot be used in infants and children.

1.5.4. Small intestinal intubation

In the proximal part of the small intestine a catheter with a balloon at the end is placed to occlude the gut lumen. Fluids from the part of the gut proximal of the occlusion can be sampled via side holes in the tube. After addition of substrate, consumed or supplied by nasogastric tube, digestion products can be sampled and further analysed.

Using this procedure the enzyme activity in the occluded proximal part of the intestine can be measured. The relation to the over all enzyme activity of the gut has not been evaluated. Due to the invasiveness of the method it is only used in research situations.

1.5.5. H_2 breath test

When lactose is not hydrolysed in the small intestine, undigested lactose will reach the colon. There the bacterial flora can ferment it. This fermentation process leads to the production of gasses including Hydrogen (H_2), methane (CH_4) and carbon dioxide (CO_2), and of lactate and short chain fatty acids, that can all be absorbed by the colonocytes. Subsequently the gasses are transported via the blood and exhaled. Since other biological processes in the human body do not produce hydrogen, the exhaled breath concentration of H_2 represents the fermentation of carbohydrate in the colon. Unfortunately the carbohydrate fermentation process in the colon is not restricted to lactose as substrate. Other carbohydrates such as fibre or undigested starch can also be subject to the similar bacterial fermentation process and lead to the same products in breath. Apart from this, many factors can influence the composition of the colonic flora, such as medication, colonic acidity and thereby the capacity to form H_2.

The result of the test is expressed as positive or negative (i.e. maldigestion yes or no) with a cut off point of 10 or 20 parts per million H_2 concentration rise above base line levels. This technique was introduced in the 1970's[25,26,27]. In understanding the mechanisms behind this test its reliability with respect to the quantity of fermented lactose can be doubted. Despite such uncertainty about results it is until today the most frequently used test to study lactose digestion.

1.5.6 $^{14}CO_2$ lactose breath test

The carbon molecules in lactose can be labelled with ^{14}C. After consumption and hydrolysis of such labelled lactose, the resulting ^{14}C -glucose and ^{14}C -galactose are metabolised and exhaled as $^{14}CO_2$. The cumulative amount of exhaled $^{14}CO_2$ is related to the hydrolysis of the substrate. However, the oxidation of the absorbed labelled monosaccharides can vary under different test conditions. Furthermore, radioactivity of ^{14}C however limits its applicability in medial research, especially in infants, children and pregnant women.

1.6 The scope of this thesis

The important nutritional contribution of dairy products to the Western diet makes that elimination of these products will have negative effects on health, unless compensatory supplements are taken. Suppletion of vitamins and calcium will be necessary and alternative protein sources have to be used. Many individuals eliminate all dairy products from their diet when they experience discomfort after milk use or when tests to measure lactose digestion have indicated lactose maldigestion. As stated before none of the available test methods has been proved to be reliable enough in accurately determining the amount of lactose that can be digested. Most test results only indicate an "all or nothing" phenomenon, while functional lactase capacity should be quantified. The availability of lactose labelled with the stable, non-radioactive isotope ^{13}C made it possible to study the digestion, absorption and metabolism of consumed lactose without the disadvantages of radioactivity. Using the stable isotope technique we were able to study the digestion of lactose in patients with reported milk intolerance symptoms. Measured digestion could be related to the occurrence of symptoms or the degree of mucosal damage in biopsy specimens.

The specific aims of the study are:

1. To develop a method to reliably measure lactose hydrolysis in humans, which can be applied in clinical practice.
2. To study the interfering factors on the $^{13}CO_2$ breath test results:
   a. The influence of variations in substrate oxidation due to different levels of exercise.
   b. The effect of variations in colonic carbohydrate fermentation on the results of the $^{13}CO_2$ breath test.
3. To evaluate glucose uptake after hydrolysis of lactose
   a. To improve the diagnostic quality of the lactose tolerance test by use of stable isotope labelled ^{13}C-lactose and measurement of the ^{13}C-glucose concentration in blood (^{13}C-lactose/^{13}C-glucose test).
   b. To develop a method that can correct for individual variations in gastric emptying rate, ileo-coecal transit time and glucose metabolism in the ^{13}C-lactose/^{13}C-glucose test.
4. Clinical applications:
   a. To study the relation between intestinal lactose digestion capacity and the occurrence of clinical symptoms.
   b. To study the effect of small intestinal mucosa damage on lactose digestion.

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Chapter 2

Non invasive detection of low intestinal lactase activity in children by use of a combined $^{13}CO_2$ /H_2 breath test

Short title: Lactose maldigestion breath test

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Abstract :

The aim of the study was to diagnose hypolactasia with a higher accuracy than with the traditional H_2 breath test. We used a combined ^{13}C-lactose $^{13}CO_2$ /H_2 breath test, which was performed in 33 patients in whom lactase activity was measured. Lactase activity was reduced in 13 cases. The sensitivity and specificity of the H_2 test were 54% and 90%; those of the $^{13}CO_2$ test 69% and 70%. False-negative results did not always occur in the same patients. In five of six patients with both tests results abnormal, lactase activity was low. In 13 of 15 patients with both tests results normal, lactase activity was normal. In 6 of 12 cases with only one test abnormal, lactase activity was low.

Conclusion: The combined H_2 /$^{13}CO_2$ breath test (sensitivity 85%, specificity 65%) is more adequate for diagnosis of hypolactasia than the H_2 breath test alone.

INTRODUCTION:

Dairy foods are important sources of high quality protein, vitamins and calcium after weaning[1]. They are relatively cheap and easy available food ingredients, which can contribute favourably to nutrition. However,many people are unable to digest lactose, the main carbohydrate in milk[2,3]. This lactose maldigestion can be caused by primary hypolactasia (rare), secondary hypolactasia (due to villous atrophy of the small intestine), or the genetically determined so called "adult type hypolactasia". This last type was traditionally predominantly found in non Western Countries, but due to increased migration it is now also increasingly present in the Western world.

Symptoms of lactose maldigestion are non-specific and consist of, among other things, abdominal pain, diarrhoea and flatulence. It has also been described as an important cause of irritable bowel syndrome in adults and children[4]. Suspicion of the diagnosis without proper confirmation can lead to an inappropriate dietary restriction of dairy products, with consequences for the intake of calcium, vitamins and energy or the use of relatively expensive enzyme preparations in addition to food.

Lactose maldigestion can be diagnosed by measuring lactase activity in a small-bowel biopsy specimen. To minimalize the use of this invasive procedure, several less invasive tests have been developed in the past decades.

The first one was based on the blood glucose response to a lactose load (Lactose tolerance test (LTT)[5]. Although original applications looked very promising, more widespread experience did not favour the LTT owing to low sensitivity and selectivity[6]. The H_2 breath test was thereafter proposed, based on the principle that non-absorbed carbohydrates will be fermented by the colonic bacterial flora, resulting in the production of, among other things,H_2 . H_2 is to some extent absorbed by the intestinal mucosa and expired via the lungs. Until now this test has been the most frequently used non-invasive test for screening carbohydrate malabsorption. However the H_2 breath test has some well known shortcomings: non-H_2-producing colonic bacterial flora , up to 20%[7,8], low colonic pH or use of antibiotics can cause false negative test results[7,9,10], as can diarrhoea in the weeks preceding the breath test[11]. Furthermore, hyperventilation and other extraintestinal influences such as the use of salicilates can disturb the test[1,12].

Because fermentation of non-absorbed carbohydrates is measured, the H_2 breath test is an indirect test of the digestive capacity of the small intestine. A more direct method can be performed by the use of labelled substrates, which are absorbed in the small intestine after hydrolysis and whose metabolised fraction can be detected as labelled CO_2 in breath. In adults ^{13}C- and ^{14}C-labelled lactose has been used for this purpose with good results[6,12-14]. Enriched ^{13}C-carbohydrate substrates make this direct test method also feasible in children, since no radioactivity is applied.

In this study, we tested the new ^{13}C-lactose breath test in paediatric patients by comparing the results with those of the simultaneously performed H_2 breath test and the standard method for diagnosis of hypolactasia: the lactase activity in small bowel biopsy specimens.
For ethical reasons we made the comparison between the results of this new breath test and the lactase activity in children, who already had to undergo a small bowel biopsy for other reasons.

MATERIALS AND METHODS:

Subjects:

Twenty-seven patients -14 boys and 13 girls- aged 11 months - 19 years (median, 4 years) participated in the study. All patients underwent an endoscopically performed small-bowel biopsy near the Treitz ligament for several clinical indications (Table 1). The biopsy was performed with the patient under sedation with midazolam (intravenous). Preceding the biopsy, a lactose $^{13}CO_2/H_2$ breath test was performed. Twenty-one patients were tested once; in six patients - five of whom have suspected celiac disease - the test was performed twice: once on a gluten-free diet and once after gluten provocation with secondary hypolactasia. The sixth patient tested twice was suspected of having inflammatory bowel disease.
The normal range of the 4-h cumulative $^{13}CO_2$ excretion (4-h cPDR) after consumption of ^{13}C -lactose in a healthy paediatric population has not been described previously. We therefore performed this ^{13}C-lactose breath test in 21 healthy school children aged 4-8 years without signs of lactose maldigestion and with a negative lactose H_2 breath test. These children were tested under the same conditions as the patients.

Table 1: Indication for Small Bowel Biopsy (SBB) in 27 patients (6 patients tested twice)

Indication for SBB	Patient no.
CD, no diet	2, 12, 16, 17, 24, 29
CD, glutenfree diet	10(=14), 11, 33, 18(=5), 25(=6), 19(=7), 32(=8), 14(=10)
CD, gluten provocation	1, 4, 5(=18), 6(=25), 7(=19), 8(=32), 30
CMA, diarrhoea on cow's milk free diet	21
CMA, treated G.lambliae enteritis, diarrhoea	15
Malabsorption	13
Abdominal Pain, positive SAT	31
Malaise, positive LH_2BT	22, 27
Short Stature, exclusion CD	26
Malnutrition, IBD	23
Malnutrition, gluten-free diet	3(=9), 9(=3)
Sucrase Isomaltase deficiency	20, 28

Lactose $^{13}CO_2$ /H_2 breath test:

After they had fasted overnight the patients were given a 20% ^{13}C-lactose solution in water in a dose of 2g/kg body weight with a maximum of 50 g. This dose and concentration are identical to those used in the classical lactose H_2 breath test[14,15]. Before substrate ingestion and half- hourly during the subsequent 4 h, breath samples were taken. End-expiratory air samples were collected in 20-ml plastic syringes with an adaptor as mouth piece, as has been described previously[16]. In very young children an anaesthetic mask was connected instead of the adaptor. Then 3 ml of the collected air was injected into a 3-ml Vacutainer tube (Terumo Europe NV, Leuven, Belgium) for H_2 determination and 15 ml into a 20-ml Vacutainer tube (Becton Dickinson Vacutainer Systems Europe, Meylan Cedec, France) for $^{13}CO_2$ /$^{12}CO_2$ analysis.

Substrate:

The substrate used was naturally labelled ^{13}C -lactose, derived from milk from cows fed cattle fodder corn for 5 weeks, produced for this study by the Netherlands Institute for Dairy Research Ede, The Netherlands. This ^{13}C-lactose powder contained 1.098% ^{13}C and had a $\delta^{13}C_{PDB}$ of -12‰. (The normal value of breath $\delta^{13}C_{PDB}$ in our population is approximately -26‰).

Breath sample analysis:

The tubes were stored at room temperature and analysed within the 1st week after the test. Under these storage conditions the quality of breath samples has been proven to remain unimpaired[17,18]. The breath samples were analysed on a HP 5880 gaschromatograph for H_2 concentration[16]. A positive test result was defined as an increase in concentration above basal H_2 values (delta H_2) of more than 20 ppm at any time point during the test period. The samples from the 20 ml tubes were analysed with isotope ratio mass spectrometry (IRMS) (Delta S/GC, Finnigan MAT, Bremen, Germany) for the $^{13}CO_2$ /$^{12}CO_2$ ratio. The cumulative percentage of the administered dose of ^{13}C expired after 4 hours (4-h cPDR) was taken as the variable for lactose digestion, as has been described previously by Hiele et al. in adults[14]. The CO_2 production rate was assumed to be 300 mmol/m^2 body surface area/h.

Small bowel biopsy technique and enzyme assay:

The small bowel biopsy specimens were endoscopically obtained by use of a modified Crosby capsule near the Treitz ligament. Enzyme activity was measured with a modified Dahlqvist method[19] and expressed in units per gram protein. Normal lactase activity was defined as an activity of more than 10U/g protein[12,13,20].

Ethical Considerations:
This protocol was approved by the Medical Ethics Committee of the Groningen University Hospital and informed consent was obtained from the parents and patients in accordance with the principles expressed in The Declaration of Helsinki.

RESULTS:

Lactase activity:
The distribution of the enzyme activity in our patients is shown in figure 1. In 13 of 33 biopsy specimens the measured lactase activity was less than 10 U/g protein (range 0.55 - 9.3 U/g). In the 20 other specimens the lactase activity was in the normal range (13.6 - 131 U/g).

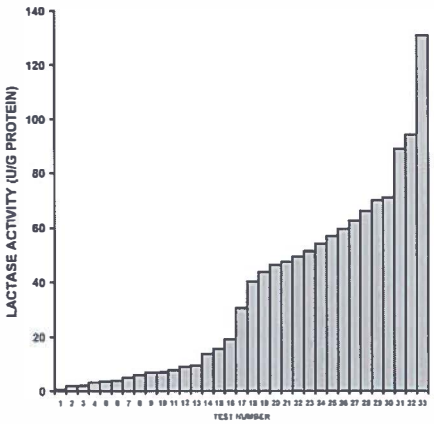

Figure 1. Lactase activity in 33 biopsy specimens.

H_2 breath test:
The H_2 breath test result was abnormal in 9 out of 33 tests. In seven of these nine tests it was accompanied by a low intestinal lactase activity. However, the low lactase activity of 6 other biopsy specimens was not detected by the H_2 breath test. This leads to a sensitivity of 54% with a specificity of 90% (Table II).

Table II: Relation between breath test results and intestinal lactase activity

Test	sensitivity	specificity	pos.pred.value	neg.pred.value
H_2	7/13=54%	18/20=90%	7/9=78%	18/24=75%
$^{13}CO_2$	9/13=69%	14/20=70%	6/15=40%	14/18=78%
Combination (at least one positive)	11/13=85%	13/20=65%	11/18=61%	13/15=87%

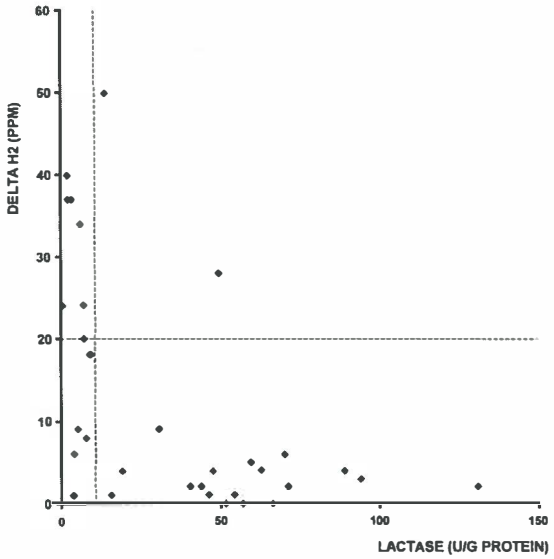

Figure 2. Relation between H_2 breath test results and lactase activity.

$^{13}CO_2$ breath test:

Because there were no literature reports defining the cut-off of the normal range of 4-h cPDR for persons in this age group, we determined the variation in healthy school children (n=21) aged 4-8 years without signs of lactose maldigestion and with a negative lactose H_2 breath test result. A mean 4-h cPDR of 21.5% (standard deviation, 4.8%) was found. Taking this mean value

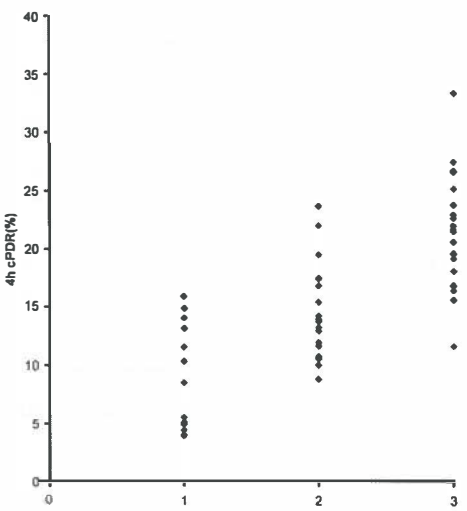

Figure 3. Four-hour cumulative $^{13}CO_2$ excretion (4-h cPDR) in patients with low lactase activity (1), patients with normal lactase activity (2), and healthy schoolchildren (3).

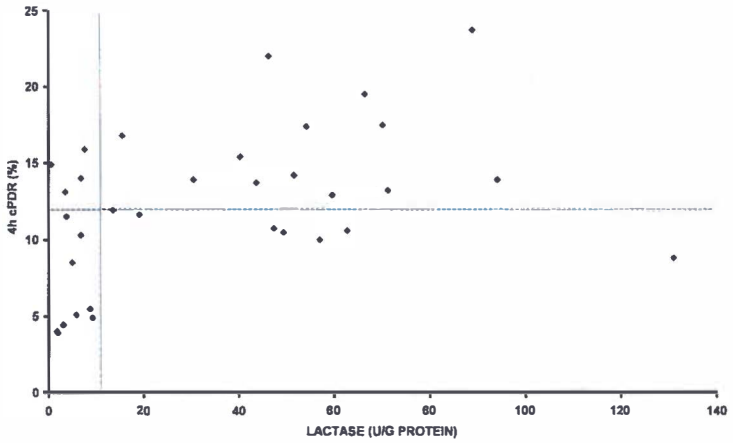

Figure 4. Relation between 4-h cumulative $^{13}CO_2$ excretion (4-h cPDR) and lactase activity.

minus two standard deviations as a reference, we defined a positive breath test as a 4-h cPDR of less than 11.9%.
The distribution of cPDR values in tests with normal and with low lactase activity is shown in Fig.4. For comparison the test results of the 21 healthy school children are also shown. In 15 of 33 clinical tests a positive $^{13}CO_2$ breath test result was found. In 9 of these 15 tests this result was related to a low lactase activity. The four other biopsy specimens with low lactase activity were missed by the $^{13}CO_2$ breath test, leading to a sensitivity of 69% and a specificity of 70% (TableII).

Combined H_2 /$^{13}CO_2$ breath test: (figure 5)
The outcome of both breath tests was not concordant in all cases. We therefore analysed the outcome of the combined H_2 /$^{13}CO_2$ breath test in relation to the lactase activity measured in the biopsy specimen. In 15 out of 33 cases both breath test results were normal. In 13 of these the breath test results were adequately related to a normal lactase activity.
In 18 out of 33 cases 1 or both breath test results were abnormal. In the six patients in whom both breath test results were abnormal, hypolactasia was diagnosed by biopsy in five cases.
In the group of 12 cases in whom only 1 of the breath test results was abnormal, in 6 a low and in 6 a normal lactase activity was found (Table III).

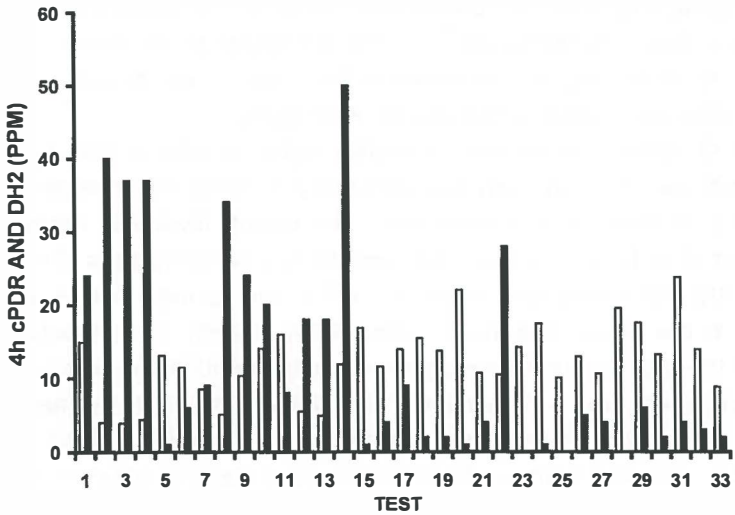

Figure 5. Relation between H_2 breath test results (closed columns) and 4-h cumulative $^{13}CO_2$ excretion (4-h cPDR) (open columns) in 33 tests.

Table III: Combination of test results in relation to lactase activity

Combination	Low Lactase	Normal Lactase
H_2 pos / $^{13}CO_2$ pos	5	1
H_2 pos / $^{13}CO_2$ neg or H_2 neg/$^{13}CO_2$ pos	6	6
H_2 neg / $^{13}CO_2$ neg	2	13

DISCUSSION:

It is known from the literature and clinical experience that a well-accepted non invasive test for diagnosis of hypolactasia, the lactose H_2 breath test, has a high proportion of false-negative results. We therefore compared the results of the new ^{13}C-lactose breath test with the intestinal lactase activity in a non-selected outpatient paediatric population with various indications for small-bowel biopsy. This lactase activity was taken as the "gold standard", although in the literature there is no consensus about the cut-off point of abnormal lactase activity levels in small-bowel biopsy specimens (<6 U/g protein[20], < 10 U/g protein[12], < 11U/g protein[14], <13 U/g protein[21], 32.1±10.1 U/g protein[22], 48±22 U/g protein[23]).

In this study biopsy specimens with a lactase activity of less than 10 U/g protein were considered to be abnormal, in agreement with most of the literature reports. It should of course be realized that the lactase activity in one small-bowel biopsy specimen cannot reflect the total lactase activity of the small bowel.

The low sensitivity of the H_2 breath test (54%) in our study is compatible with results reported in the literature[13,24,25]. The low breath H_2 excretion can be caused by a non-H_2-producing colonic bacterial flora and/or extraintestinal influences such as hyperventilation or the use of medication.

For the $^{13}CO_2$ breath test we found a slightly higher sensitivity (69%) for detection of hypolactasia. This relatively low sensitivity is, however, strongly determined by the cut-off level for a positive test. The cut-off level was determined in a population of 21 healthy school children who had no symptoms of hypolactasia. Surprisingly, the distribution of the 4-h cPDR and its mean value were not the same as in the group of patients with biopsy proven normal lactase activity. Although the test conditions were planned to be identical to those of the patient population, there was a higher level of physical activity in the healthy school children compared to the patients during the test. This might have affected the 4-h cPDR results, as it is known from the literature that the oxidation of exogenous carbohydrate substrate is increased during exercise[26]. The apparent difference between the two groups, however, is not statistically significant (*t* test).

The false-negative results in the clinical study, a problem in both breath tests, did not always occur in the same patients. The causes underlying these false-negative results therefore appear to be different. In the H_2 breath test the result of colonic fermentation of undigested lactose is important, whereas in the $^{13}CO_2$ test the metabolized result of absorbed glucose and galactose is measured.

Analysis of factors that might interfere with these test results, such as colonic CO_2 production by fermentation of malabsorbed carbohydrate and variations in glucose oxidation rate will be the subject of our further studies. Adjustment of test conditions can presumably improve the sensitivity of the $^{13}CO_2$ breath test. On the basis of the disconcordant occurrence of false-negative H_2 and $^{13}CO_2$ breath test we considered the combination of tests as an alternative technique to detect low lactase activity. For this purpose the finding of at least one of two breath tests positive was considered an indicator of hypolactasia. As a result a sensitivity of 85% with a specificity of 65% was found.

In the "double-positive group" 5 of 6 patients did indeed have a low lactase activity (83%), whereas in the "double-negative group" a normal lactase activity was found in 13 of 15 tests (87%).

In the "disconcordant breath test group" a low lactase activity was found in 6 of 12 tests (50%).

If we had decided to perform a biopsy only in those patients with disconcordant breath test results the number of biopsies could be reduced from 33 to 12 (64% reduction).

The combination of breath test results matched with the lactase activity of the intestinal biopsy specimen in 30 of 33 tests (91%). As a result of this policy only two patients with low lactase activity would have been missed, whereas one

patient would have been inadequately diagnosed as having low intestinal lactase activity.

We conclude that the ^{13}C -lactose breath test with measurement of $^{13}CO_2$ and H_2 response is better related to the intestinal lactase activity than the traditionally used lactose H_2 breath test. The interpretation of the combination of test results ($^{13}CO_2$ and H_2) can reduce the number of biopsies necessary for detection of hypolactasia with more than 60%. This makes this new test very suitable in patients with non-specific abdominal complaints, which might be related to lactose maldigestion. The test is also very suitable for population studies, which have until now only been performed by use of the LTT and H_2 breath test with the already discussed disadvantages.

This test can also be used for determination of the optimal time for biopsy in patients undergoing gluten provocation because of suspected celiac disease, as it is known that the timing of biopsy can be hampered by a very quick or slow clinical reaction on provocation in which the histological changes of the mucosa are not manifest at the same time.

Acknowledgement

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Chapter 3

^{13}C-Carbohydrate breath tests: impact of physical activity on the rate-limiting step in lactose utilisation

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ABSTRACT

Background

$^{13}CO_2$ breath test can be used to monitor carbohydrate digestion in the small intestine. However, after ingestion of ^{13}C- substrates $^{13}CO_2$ excretion in breath originates from two sources: a digestive/oxidative fraction, derived from the small intestine, and a fermentation fraction, derived from undigested carbohydrate spill-over in the colon. In this study, the determinants of the digestive/oxidative fraction were analysed in order to improve the sensitivity/ specificity of the ^{13}C-carbohydrate breath test.

Methods

^{13}C-carbohydrate breath test were performed in healthy adults using ^{13}C-lactose, pre-digested ^{13}C-lactose, ^{13}C-glucose and ^{13}C-galactose as substrates. The effect of exercise (bicycling, 50 W), increasing the metabolism of digested/absorbed substrate, on the outcome of the test was analysed.

Results

In rest, no difference was observed in the 4-h cumulative percentage dose recovered in breath (4-h cPDR) after administration of glucose, pre-digested lactose and lactose, which were 20.3 ± 4.5 %, 19.2 ± 5.5 % and 19.9 ± 4.9 %, respectively. The $^{13}CO_2$ excretion rate after ^{13}C-galactose consumption was significantly slower than after ^{13}C-glucose consumption. Exercise increased 4-h cPDR of ^{13}C-glucose significantly: 76.0 ± 1.0 % vs. 22.7 ± 2.3 %. This effect was also observed using ^{13}C-lactose as substrate: 66.1± 6.2% vs. 19.6 ± 3.9%. One subject had non-symptomatic lactose malabsorption indicated by a positive H_2 breath test. The $^{13}CO_2$ breath test of this subject in rest was indistinguishable from that of the others (4 h cPDR 16.6 vs. 19.6 ± 3.9 %), whereas the test was clearly indicative during exercise (4 h cPDR 20.5 vs. 66.1 ± 6.2 %).

Conclusion

In healthy volunteers in rest, glucose oxidation is the rate-limiting step in lactose conversion into $^{13}CO_2$.Increase of metabolism (for instance, by exercise) can shift this step to intestinal hydrolysis of lactose, making the ^{13}C-lactose breath test more sensitive.

INTRODUCTION

Hypolactasia can be diagnosed by the H_2 breath test or a combined $H_2/^{13}CO_2$ breath test when ^{13}C-lactose is used as a substrate[1,2]. In a previous study we observed a high percentage of non-congruent test results between the H_2 and the $^{13}CO_2$ breath tests[2]. H_2 is derived from fermentation of maldigested lactose, while $^{13}CO_2$ can be derived both from digested and metabolised lactose in the small intestine and from maldigested lactose which is spilled over into the colon[3]. Breath test results deviating from those of the gold standard, being the lactase activity measurements in biopsy material, are mainly false negatives, leading to a low sensitivity[1,2,4-7]. False-negative H_2 breath test results can be explained by the presence of non-hydrogen-producing colonic bacteria, hydrogen-consuming bacteria, and colonic faecal flora alterations due to extreme pH variations, or the use of antibiotic drugs[8-12]. Factors responsible for false-negative $^{13}CO_2$ results are not well studied. A false negative $^{13}CO_2$ test could be caused by high $^{13}CO_2$ production due to alternative sources, such as the colon. Because in rest the total $^{13}CO_2$ excretion, originating from digested and absorbed substrate, is relatively low (<25% 4-h cPDR), we speculated that increasing the contribution of the digestive/oxidative fraction would improve the sensitivity of the breath test. Therefore we studied the rate-limiting step in the conversion of ^{13}C-lactose to $^{13}CO_2$ and whether this step was positively influenced by exercise.

MATERIALS AND METHODS

Materials

^{13}C lactose was produced in collaboration with the Dutch Dairy Research Institute (NIZO, Ede, The Netherlands). For this purpose cows were fed cattle fodder corn for a period of 5 weeks. Lactose was isolated from the milk by standard procedures. The ^{13}C abundance was 1.098 % ($\delta\ ^{13}C_{PDB}$ (PeeDee-Belemnite Limestone) -12 ‰). Chemical purity after filtration of the lactose solution was 95%. Commercial dextrose (corn glucose) was used as the ^{13}C- glucose substrate (batch to batch ^{13}C abundance variation 1.098 - 1.099 %). D [1- ^{13}C] galactose was purchased from Isotec Inc, Miamisburg, Ohio, USA and administered as a mixture with unlabeled D-galactose (Aldrich Chemical Co ltd, Gillingham, UK). Breath samples were collected in 10-mL exetainers (LABCO limited, High Wycombe, UK). Lactase to pre-digest lactose (Kerulac®) was kindly donated by Artu Biologicals N.V., Lelystad, The Netherlands. Kerulac contains β-D-galactoside galactohydrolase from *Aspergillus oryzae*.

Methods

Subjects

All subjects were healthy adults (age 19-29 years, male/female ratio 0.69) of Northern European background. Their medical history did not reveal serious

gastrointestinal complaints nor recent antibiotic treatment. Although average daily intake of milk varied from < 1 to 4 glasses per day, none of the volunteers reported complaints after milk intake. For testing the various substrates, different individuals were involved: lactose (n=25), pre-digested lactose (n=23), glucose (n=43), and galactose (n=10). For the exercise experiments, the individuals underwent the breath test twice, once at rest and once during exercise (glucose: n = 5; lactose: n = 7). There were at least 7 days between tests. The study was approved by the Ethics Committee of the University Hospital Groningen and conducted in conformity with the Declaration of Helsinki.

Study protocol

The participants were asked to refrain from consuming cane sugar, corn, corn products, and pineapple during the last two days before the test since these items are naturally enriched in ^{13}C. They arrived at the clinical department at 08.00 h after an overnight fast of at least 12 h. During the fasting period only non-caloric drinks (water, tea and coffee without sugar or cream) were allowed. Two baseline breath samples were collected before ingestion of the substrate. After ingestion a breath sample was collected every 30 min up to 4 h. For the rest experiments a dose of 40 g ^{13}C substrate was administered orally dissolved in 200 ml water. The test substrates for the study of the exercise study were 40 g naturally enriched ^{13}C-glucose and 80 g naturally enriched ^{13}C-lactose, the latter dissolved in 400 ml water. Thus, two sets of experiments were carried out: the carbohydrate test was performed once in rest and once during exercise by bicycling at 50 W (Ergometer, Lode, Groningen, The Netherlands). The test periods lasted 4 h. During exercise the test substrate was administered to the bicyclist after a 15-min warming up period. The total CO_2 production was determined by indirect calorimetry (Oxycon, Jaeger Benelux B.V., Breda, The Netherlands) by measuring at 30-min intervals during a period of 5 min. For estimation of maximal $^{13}CO_2$ excretion 100 W exercise was used.

Pre-digestion of lactose

Lactose was pre-digested by treatment with lactase (Kerulac) for 24 h at 4°C resulting in glucose and galactose. Twenty drops of Kerulac were added to 40 g lactose dissolved in 200 ml water after adjustment of the pH to 6.8. The reaction efficiency was tested by gas chromatographic analysis[12] and found to be >98%.

Measurements

Breath H_2 concentration was determined by gas chromatography[13]. H_2 excretion in breath is expressed as μmol/min in order to facilitate comparison of results in rest and during exercise. ^{13}C abundance of breath CO_2 was determined by continuous flow Gas Isotope Ratio Mass Spectrometry (GIRMS) (Breath MAT, Finnigan MAT, Bremen, Germany) measuring the $^{13}C/^{12}C$ ratio as $\delta\ ^{13}C$ vs. PeeDee-

Belemnite Limestone ($\delta^{13}C_{PDB}$) in ‰. After conversion of $\delta^{13}C_{PDB}$ to ^{13}C abundance (%), the excretion rate of ^{13}C in breath as percentage dose/h (PDR) was calculated based on a total breath CO_2 excretion rate of 300 mmol/m^2 body surface area/h in rest or total breath CO_2 excretion measured with indirect calorimetry during exercise experiments. The cumulative % dose ^{13}C recovered in breath (cPDR) was calculated thereafter.

Statistical analysis

Data are expressed as mean ± standard deviation. In the experiments in rest, differences in PDR and cPDR between substrates were tested using the one-tailed Student *t* test applied to data obtained at 60, 120, 180 and 240 min. The hypothesis was that response to glucose would be greater than response to galactose and that response to pre-digested lactose would be greater than response to lactose. A *P* value < 0.05 was considered statistically significant.

RESULTS

To analyse the rate-limiting step in ^{13}C-lactose utilisation, the breath ^{13}C excretion curves for lactose and pre-digested lactose were compared as judged by the time point of maximum PDR, the maximum PDR value and the cPDR at each time point (Fig 1). At no time point tested was a statistical difference obtained. Comparing the excretion curves for glucose and galactose, we see that the galactose curve is shifted to a later time point, indicating a delay in oxidation (Fig.2). At t = 60, 120, and 240 min the ^{13}C excretion rates were significantly different ($P < 0.002$). cPDR values were also significantly different at all time points ($P \leq 0.036$). No time shift is observed when the curves for pre-digested lactose and glucose are compared and no difference is observed between the oxidation rates of lactose and glucose (Fig 3.). After 4 h the cPDR values are not

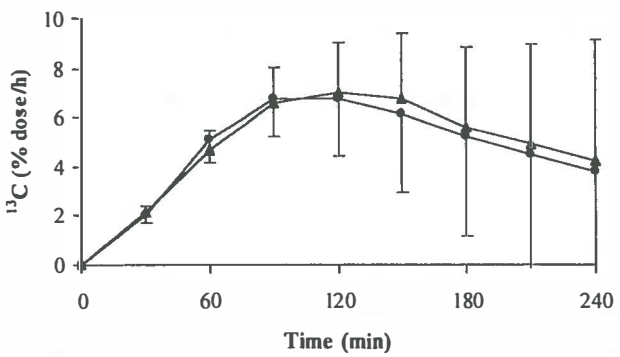

Figure 1. ^{13}C excretion rate in breath after ingestion of 40 g ^{13}C-lactose (▲-▲;n=25) or pre-digested ^{13}C-lactose (●-●; n=23) in healthy volunteers.

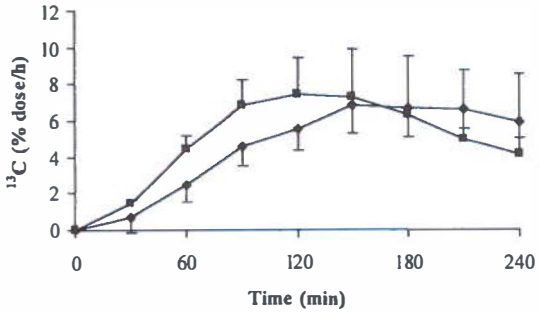

Figure 2. ^{13}C excretion rate in breath after ingestion of 40 g ^{13}C- galactose (◆-◆; *n*=10) or ^{13}C-glucose (■-■;*n*=43) in healthy volunteers.

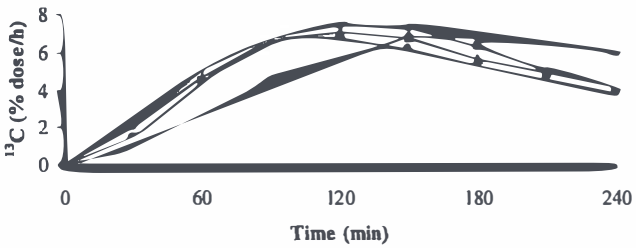

Figure 3. ^{13}C excretion rate in breath after ingestion of 40 g ^{13}C- galactose (◆-◆; *n*=10), ^{13}C-glucose (■-■; *n*=43), ^{13}C-lactose (▲-▲; *n*=25) or pre-digested ^{13}C-lactose(●-●; *n*=23) in healthy volunteers.

significantly different (Table I). None of the subjects revealed an increase in H_2 concentration in breath exceeding 20 ppm over the baseline value during the 4-h test period. These data indicate no differences in the overall processing of lactose, pre-digested lactose and glucose. Moreover, the overall processing of galactose seems to be facilitated by glucose.

From these data it can be derived that lactose digestion in rest is not the rate-

Table I: Cumulative ^{13}C recovery 4 h after ingestion of substrate

	4-h cPDR	*P*
Glucose	20.3 ± 4.2	
Galactose	18.2 ± 2.4	0.036
Pre-digested lactose	19.2 ± 5.5	NS
Lactose	19.9 ± 4.9	NS

Cumulative % dose recovered in breath (cPDR) 4 h after ingestion of 40 g ^{13}C-lactose or lactose digestion products in rest.

P= signifance of difference to the glucose response.

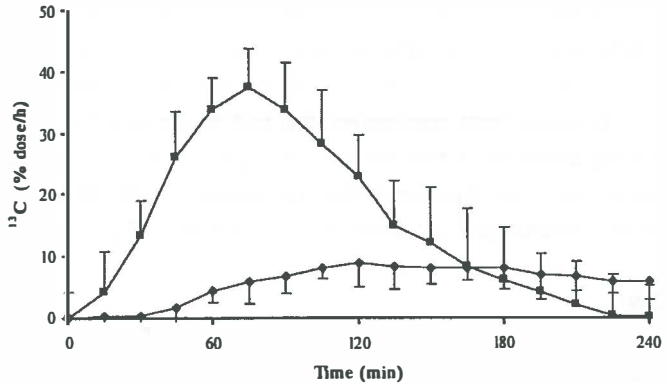

Figure 4. $^{13}CO_2$ exctretion rate in breath after ingestion of 40 g ^{13}C-lactose in rest (◆-◆?) and during 50 Watt exercise (■-■) in healthy volunteers (n=5).

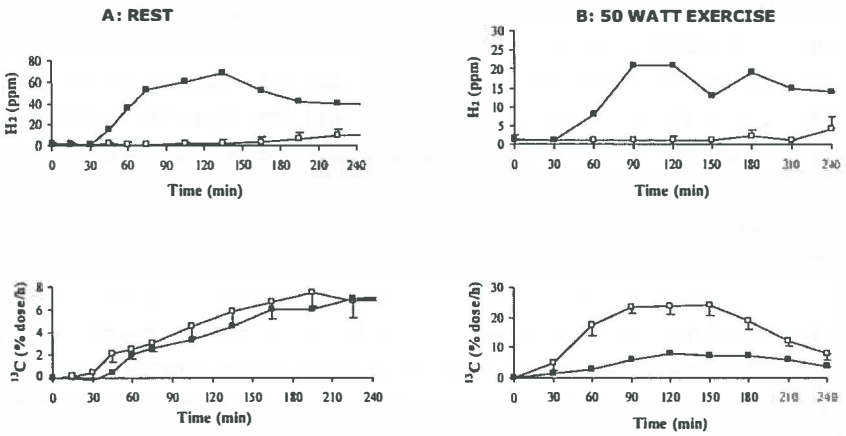

Figure 5. H_2 excretion and $^{13}CO_2$ excretion rate in breath after ingestion of 80 g ^{13}C-lactose in rest (A) and during 50W exercise (B). A comparison is made between healthy volunteers (□-□; *n*=6) and subject A (■-■), a (non-symptomatic) lactose maldigester.

limiting factor in the overall utilisation, but one of the processing steps of its hydrolysis products, most likely oxidation. To study this effect, the influence of physical exercise was investigated. Fig.4 illustrates the influence: a clear increase in $^{13}CO_2$ production rate (4-h cPDR) after consumption of glucose is observed during 50-W bicycling (4-h cPDR 76.0 ± 1.0% vs. 22.7 ± 2.3%) followed by a further increase to 90% during 100-W bicycling (data not shown). This increase is probably caused by a higher oxidation rate of glucose. Subsequently, the effect of physical exercise on lactose utilisation was studied in a second group of volunteers. To challenge lactose-digesting capacity, a dose of 80g lactose was used. Only one subject (A) revealed a distinct increase in breath H_2 in rest (maximum 34.6 vs. 4.4 ± 2.1 μmol min^{-1}). A further increase in the test dose of lactose, in order to demonstrate lactose maldigestion in all subjects, was

considered inappropriate. The $^{13}CO_2$ excretion in breath of subject A was not significantly different from the others during the rest condition (4-h cPDR 16.6 vs. 19.6 ± 3.9%) (Fig. 5a). During the 50-W bicycling experiment the cumulative recovery of ^{13}C in breath was increased in 6 out of 7 subjects, as was predicted by the glucose experiment (4-h cPDR 66.1 ± 6.2%), but not in subject A (20.5%) (Fig.5b). During exercise, however, the increased breath H_2 production in this subject persisted (maximum 22.0 vs. 9.5 ± 4.1 ppm) (Fig. 5b).

DISCUSSION

The excretion of $^{13}CO_2$ in breath after consumption of ^{13}C-labelled carbohydrates can be derived from the substrate, which is digested and absorbed in the small intestine and subsequently oxidized, but also from the fermentation of the maldigested substrate in the colon. In a recent study, we demonstrated that this latter source can have a considerable effect on the overall $^{13}CO_2$ production, leading to false negative results[3]. Therefore, we analysed the factors influencing the digestion/oxidation pathway, relevant for small intestinal lactase activity, in order to improve the sensitivity/specificity of the $^{13}CO_2$ lactose breath test.

When the $^{13}CO_2$ breath tests using lactose and pre-digested lactose under standard conditions were compared, no significant differences were observed, which indicates that lactose hydrolysis is not the rate-limiting step in the overall process of lactose utilization in healthy volunteers. From comparison of the glucose, lactose and pre-digested lactose breath tests, it can be concluded that other factors influencing the $^{13}CO_2$ production rate, such as gastric emptying and small intestinal transit time, are similar for all three substrates. Galactose processing was clearly slower than that of glucose. This is observed at a dose of 40 g. However, when galactose and glucose are administered together at half the dose of each carbohydrate (pre-digested lactose), no time delay is seen when compared to an equivalent dose of glucose alone. This suggests that the observed time delay in processing is dose-dependent or that the presence of glucose is of importance, for instance, in facilitating faster intestinal galactose absorption. A similar observation is described for intestinal absorption of fructose[14,15]. The mechanism of this interaction is still unclear.

Because intestinal hydrolysis seemed not to be the rate-limiting step in overall processing of lactose, we focussed on another possible factor, the glucose oxidation rate. Indeed, it was demonstrated that physical exercise increased the overall recovery of $^{13}CO_2$ after consumption of ^{13}C-glucose, which suggests increase in oxidation rate. Similar effects of physical exercise were obtained before using ^{13}C-lipids as substrate[16]. Subsequently, the effect of physical exercise on the digestion of ^{13}C-lactose was investigated. To challenge the digestive system we used a dose of 80 g of lactose and expected that the rate-limiting step would occur at the hydrolysis step. In only one out of seven volunteers could we demonstrate this. In the resting situation in this person the H_2 excretion

was increased, which indicates lactose maldigestion at this dose.$^{13}CO_2$ excretion in this subject was not significantly different from the rest of the group. During exercise, however, $^{13}CO_2$ excretion in this person did not increase. H_2 excretion remained elevated under these conditions. Exercise did not change breath H_2 excretion quantitatively. Small intestinal transit time measured by the onset of H_2 excretion in breath was also unchained. This suggests that exercise has no major effect on small intestinal transit time under these circumstances. A glucose breath test in the lactose-maldigesting subject was negative (data not shown) indicating that bacterial overgrowth in the small intestine was not the cause of the deviating results of the lactose breath test in this subject.

These data imply that in rest only a small part of the digested ^{13}C-lactose is oxidised to $^{13}CO_2$. Therefore, digestion of ^{13}C-substrate is not the rate-limiting step in the overall process. During exercise, however, in a subject with hypolactasia (shown by a positive H_2 test), substrate availability is insufficient and the lactose digestion rate becomes rate- limiting. In this situation substrate oxidation more closely reflects substrate digestion. The implication of this finding is that exercise improves the discriminative value of the lactose $^{13}CO_2$ breath test. The level of exercise has to be optimised so that it can be standardised to create maximum discrimination at an early time point. In subject A, 50W exercise allowed discrimination at a time point as early as 1h after ingestion of the substrate. As such, exercise at low activity levels allows better discrimination and a drastic reduction of the time period necessary for sample collection. However, standardized exercise may be impractical for diagnostic purposes in (small) children, who make up a very important target group for diagnosis of lactose maldigestion. For these cases, the search towards alternative techniques should be continued.

From this study it may be concluded that in the lactose- $^{13}CO_2$ breath test in the commonly used rest condition, glucose oxidation instead of lactose hydrolysis is the rate-limiting step in the overall utilisation process. Diagnosis of maldigestion using this technique appears to be limited to severe cases in which substrate digestion provides insufficient glucose for normal energy production in rest. In mild hypolactasia, diagnosis is improved by increasing the level of glucose oxidation and the physical activity level. In this situation the rate-limiting step shifts from glucose oxidation towards intestinal lactose hydrolysis.

Acknowledgements:

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CHAPTER 4

Variations in colonic H_2 and CO_2 production as a cause of inadequate diagnosis of carbohydrate maldigestion using breath tests

Short title: colonic gas production in carbohydrate maldigestion

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ABSTRACT:

Background: Lactose maldigestion is usually diagnosed by means of the H_2 breath test. When ^{13}C-lactose is used as substrate, a $^{13}CO_2$ breath test can be performed simultaneously. In an earlier publication we described the relation between both the H_2 and $^{13}CO_2$ exhalation in breath and the measured intestinal lactase activity after consumption of ^{13}C-lactose. We found a discrepancy between both breath test results in 36% of the cases. To investigate the possible cause of these incongruous breath test results we studied gas production from carbohydrate in the colon, using ^{13}C-lactulose as a non-absorbable substrate.

Methods: experiments were performed in 21 subjects, applying 5 different dosages of ^{13}C-lactulose. Repeatability studies were performed in six of these subjects, using 10 g substrate (3 tests with 1-week intervals).

Results: Both the H_2 and $^{13}CO_2$ excretion in breath varied strongly interindividually and intraindividually after consumption of ^{13}C-lactulose. In both cases no dose-response relation was observed. A significant positive linear relationship was found between H_2 and $^{13}CO_2$ exhalation ($r = 0.45$, $p<0.005$). Extrapolation of these results to ^{13}C-lactose breath tests indicates that the colonic contribution of $^{13}CO_2$ production to the total $^{13}CO_2$ excretion in breath varies but is on the average large enough to cause false negative $^{13}CO_2$ breath test results.

Conclusions: Excretion in breath of $^{13}CO_2$ produced in the colon during a ^{13}C-lactulose breath test correlates with the breath H_2 excretion. This could explain the occurrence of false-negative $^{13}CO_2$ lactose breath tests when colonic gas production is high and false-negative lactose H_2 breath test results when gas production is low. It can also explain the improved sensitivity of the combined $H_2/^{13}CO_2$ lactose breath test compared with both breath tests alone.

INTRODUCTION:

Since 1978 H_2 breath tests have been widely used for non-invasive detection of carbohydrate maldigestion[1-3]. However, it is known that the net H_2 production in the colon (that is, production minus consumption) varies interindividually and periodically[4-6].The prevalence of so-called "non H_2 producers" varies considerably in different publications[7-10]. The H_2 producer state of an individual is usually studied by means of breath tests with lactulose (4-0-β-D-galactopyranosyl-D-fructose) as a substrate[11]. This synthetic carbohydrate is not absorbed in the small intestine and is fermented in the colon. This fermentation process and subsequent metabolic processes result in the production of different gasses (H_2 , CO_2, CH_4) , which can to some extent be reabsorbed by the colonic mucosa and exhaled in breath[12,13].

In a previous study[14] we showed that a combined H_2 /$^{13}CO_2$ lactose breath test has a higher sensitivity for detection of hypolactasia than the traditionally used H_2 breath test. We concluded that the $^{13}CO_2$ measurement directly from digested ^{13}C-lactose had an additive value, because it results from the absorption process instead of colonic fermentation, as in the case of the H_2 test. However, in case of lactose maldigestion a fraction of the substrate dose reaches the colon and will be fermented, probably also resulting in $^{13}CO_2$ production. This colonic $^{13}CO_2$ production could lead to false-negative $^{13}CO_2$ breath test results. To get insight into the contribution of the colonic production to the total $^{13}CO_2$ excretion in breath, we studied the $^{13}CO_2$ production in the colon using different amounts of carbohydrate. For this purpose we used ^{13}C-lactulose as a substrate, which is not digested or absorbed in the small intestine and which enabled us to study colonic $^{13}CO_2$ and H_2 production simultaneously.

MATERIALS AND METHODS:

Subjects:

In 21 healthy adults (16 women and 5 men, age range 20 - 52 y),a total of 43 breath tests were performed using different dosages of ^{13}C-lactulose. All persons were apparently healthy and had not been taking antibiotics during the two weeks before the test. Extensive consumption of naturally ^{13}C-enriched foods for 2 days before the test was omitted, as was smoking before and during the test. Verbal informed consent was obtained from all tested subjects. The study was approved by the Ethical Committee of the University Hospital Groningen and conducted in conformity with the Declaration of Helsinki.

Substrate:

The substrate used was lactulose naturally enriched in ^{13}C. This material was obtained by isomerisation of lactose (4-O-β-D galactopyranosyl-D-glucose) to lactulose (4-O-β-D galactopyranosyl-D-fructose). The lactulose was recovered

by crystallisation first from methanol and thereafter from ethanol. In accordance with Good Medical Practice procedures the product was purified by final recrystallisation from distilled water and pharmaceutical ethanol. The end product was chemically 89% pure. The major impurities were lactose (6%) and other sugars (2%). Water and ethanol accounted for 0.2% and 0.6% respectively. The starting material lactose originated from milk of cows fed corn fodder[14]. The ^{13}C content was 1.098%. The ^{13}C content of the end product lactulose was 1.095%. Since the ^{13}C content of breath CO_2 in the Dutch population is around 1.082%, the lactulose was considered sufficiently enriched in ^{13}C to study its fermentation to products leading to $^{13}CO_2$ exhalation in breath. The substrate was consumed as a 20% solution in water.

Test protocol:
After an overnight fast (>10h) the subjects arrived at the test unit and produced three basal breath samples. After substrate ingestion breath samples were collected every 30 minutes for a period of 4 h for measurements of ^{13}C abundance in CO_2 and H_2 concentrations. During the test all subjects were quietly seated; from two hours after substrate ingestion consumption of non-caloric drinks was allowed. The protocol was identical to that of the previous described ^{13}C-lactose breath test[14]. The tested subjects consumed different doses of substrate. To study the inter- and intra-individual variation of the test results, one test, with a substrate dose of 10 g, was performed 3 times in 6 of the 21 subjects, at one week intervals.

Breath sampling and analysis:
End-expiratory breath samples were collected via a straw in 10-ml tubes (Exetainers, Labco Limited, High Wycombe, UK). The hydrogen concentration was determined first by gas chromatography applying an HP5880 gas chromatograph[15]. In the same samples the ^{13}C abundance of CO_2 was determined by continuous flow isotope ratio mass spectrometry (TracerMAT, Finnigan MAT GmbH, Bremen, Germany). The increase in hydrogen concentration over the baseline value before intake of lactulose was calculated ($\Delta[H_2]$ in ppm). The 4-h cumulative hydrogen excretion (4-h cH_2) was calculated as the area under the curve of the $\Delta[H_2]$ concentration time curve. ^{13}C abundance of CO_2 was determined relative to Pee Dee Bee limestone (PDB) as the $\delta\ ^{13}C_{PDB}$ value. This value is converted to the atom percent ^{13}C. The atom percent excess (APE) at time point t is obtained by subtracting the base-line value at time 0. Applying APE to a basal CO_2 production rate of 300 mmol/h/m^2 body surface results in an exhalation rate of ^{13}C. The cumulative percentage of the dose of ^{13}C that was recovered after 4 hours (4-h cPDR) was calculated as the area under the curve of the ^{13}C exhalation (% dose/h) time curve.

Statistics and calculations:

The total exhalation of H_2 during the 4 h after substrate ingestion (4-h cH_2) was calculated (ppm x h). A positive breath test result was defined as a H_2 concentration increase of 20 ppm or more above the base line level at any time point during the test period.

The total ^{13}C excretion as $^{13}CO_2$ during the test period, as a percentage of the ^{13}C dose consumed (4-h cPDR) was multiplied by the dose. This value represents the absolute quantity of dose that is converted to $^{13}CO_2$ and recovered in breath (4-h cDR). For the $^{13}CO_2$ calculations a basal estimated CO_2 production of 300 mmol/m² body surface was used as described previously[14].

The relation between the consumed dose and the recovery of H_2 and $^{13}CO_2$ in breath was studied by means of the linear correlation coefficient (r). The statistical significance of this coefficient was tested with the two-tailed students t test. The relation between H_2 and $^{13}CO_2$ exhalation in the tested individuals was analysed by use of the Spearman rank correlation test. A value of $P < 0.05$ was considered to be statistically significant.

To study the reproducibility of test results, six individuals were tested three times with an identical test dose of 10g ^{13}C-lactulose. The mean value, the standard deviation (s) and the coefficient of variation (CV) of the three subsequent H_2 breath test results for each individual was calculated. The mean s and mean CV of all six individuals were considered the variables to describe intraindividual variation. The s and CV of the means of the six individuals for each of the three tests were also calculated (interindividual variation) (Table I).

Table I: Variation in 4h cH_2 and 4h cDR in 6 individuals (a-e) after consumption of 10 g ^{13}C lactulose in 3 repeated sessions with one week intervals.

4h cH_2									
	person								
test	a	b	c	d	e	f	mean	s	CV %)
1	143	48	147	203	36	218	132.5	69.6	52
2	136	76	102	272	14	254	142.3	92.9	65
3	-25.8	77	107	307	63	310	139.7	126	19
mean	84.4	67	118.7	260.7	37.7	260.7			
S	78	13.4	20.1	43.2	20	37.9			
CV (%)	92	20	17	17	53	15			

4h cDR									
	person								
test	a	b	c	d	e	f	mean	s	CV (%)
1	2.31	0	2.15	3.56	2.94	4.26	2.54	1.34	53
2	0.24	2.27	3.51	3.79	4.2	5.81	3.3	1.72	52
3	0.54	0.97	3.89	3.26	1.03	1.72	1.9	1.25	66
mean	1.03	1.08	3.18	3.54	2.72	3.93			
S	0.9	0.93	0.75	0.22	1.3	1.69			
CV (%)	87	86	23	6	48	43			

The same procedure was used to evaluate the $^{13}CO_2$ results of these six individuals.

^{13}C-Lactose fermentation model derived from ^{13}C-lactulose data

To extrapolate the $^{13}CO_2$ excretion rate in breath derived from colonic fermentation of a known amount of carbohydrate substrate to the total recovery of $^{13}CO_2$ in ^{13}C-lactose digestion tests, we created a calculation model . For this model we assumed a mean 4-h cPDR of 21.5% (*s,* 4.8%) after consumption of 2g/kg body weight (with a maximum of 50 g) of ^{13}C-lactose in lactose digesting subjects, as has been described previously[14] With the substrate dose of 50 grams, assuming 100% lactose digestion and 100% absorption of glucose and galactose, this means that 21.5% of the dose is oxidized to CO_2. The remaining 78.5% is consequently retained in the body stores or metabolized to other products within the 4-h test period.

We simulated the effect of increasing lactose maldigestion rates on the CO_2 production in the colon by use of ascending doses of ^{13}C-lactulose. Fermentation in the colon of 40 g ^{13}C-lactulose represents a situation of a lactose maldigestion of 80% (40 of 50g lactose reaching the colon) in this model. The mean measured 4-h $^{13}CO_2$ (4-h cDR) recovery for a tested ^{13}C-lactulose dose (g) was added to the metabolic 21,5% $^{13}CO_2$ recovery of the equivalent fraction of digested lactose, the sum of doses always being 50g carbohydrate (Fig 4). This implies that for a situation with 80% lactose maldigestion a 4-hDR value of 2.15 g results from oxidation of digested substrate (lactose), whereas a value of 3.8 g results from fermentation of non-digested substrate (lactulose).

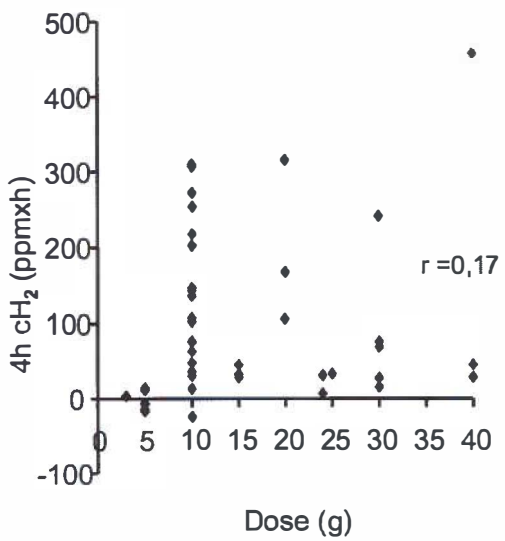

Figure 1: Relation between consumed ^{13}C-lactulose dose and 4h cH_2 response.

RESULTS:

To study the colonic gas production after ^{13}C-lactulose consumption, first the relation between the ^{13}C-lactulose dose and the 4-h cH_2 (ppm) was investigated. In Fig. 1 a large variation in 4-h cH_2 is shown, without a significant dose-response relationship (r = 0.17, NS). Furthermore, no dose-response correlation was found with colonic $^{13}CO_2$ production: the 4-h DR related to the lactulose dose (Fig. 2) showed great variation, without significant correlation (r= 0.25, NS). However, when the quantitative exhalation of both colonic gasses, H_2 and CO_2, was analysed the relationship between 4-h cH_2 and 4-h DR showed a positive correlation (r= 0.44, P<0.005), which is shown in Fig 3.

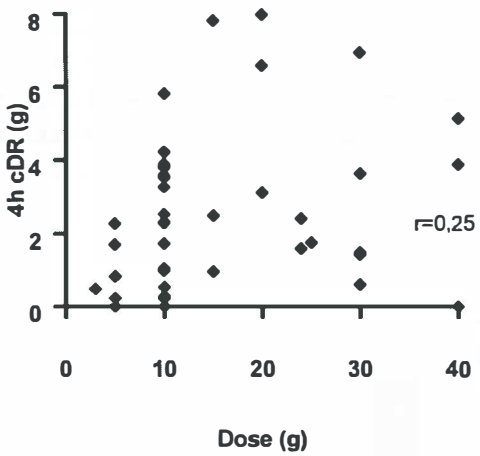

Figure 2: Relation between consumed ^{13}C-lactulose dose and 4h cDR.

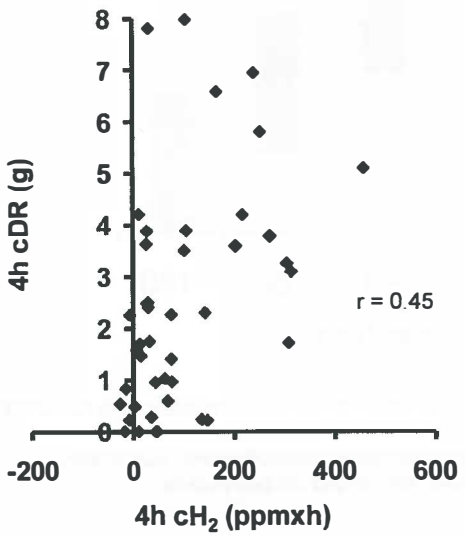

Figure 3: Relation between 4 h cH_2 and 4h cDR in 43 simultaneously performed tests.

To analyse the variability, the mean CVs for the H_2 and $^{13}CO_2$ response in six healthy individuals in three consecutive tests with 10g ^{13}C-lactulose were studied. These results are shown in table I. For the H_2 response the mean interindividual CV was 45.3% (*s*, 19.4), and the intraindividual CV was 35.7% (*s*,28.4%). For the 4-h cDR these results were 57% (*s*, 6.4) and 48.8% (*s*, 29.9) respectively. In the repeated studies with 10 g lactulose one subject (A) was classified as a non-H_2 producer (H_2 concentration rise above the base line level less than 20 ppm) once, while in the two other tests this individual had a positive H_2 test. The other five subjects were H_2 producers in all three tests.

In table II the mean 4-h cDR and SDs for five dose categories of ^{13}C-lactulose are given. The 4-h cDR values are added to the calculated 4-h cDR of the complementary dose of digested lactose in the model calculations. For instance, fermentation of 20 g ^{13}C-lactulose gives a 4-h cDR of 5.1 g. Extrapolated to an ingested dose of 50g of lactose this would mean that 30 g (60%) of the substrate is digested and metabolized, leading to a metabolic 4-h cDR of (21.5% x 30g =) 6.5 g. The total 4-h cDR for this calculation model is consequently 11.6 g. In this situation 44% of the exhaled $^{13}CO_2$ originates from colon fermentation instead of digested and metabolized substrate. In Fig.4 the results of the calculations with this model are presented.

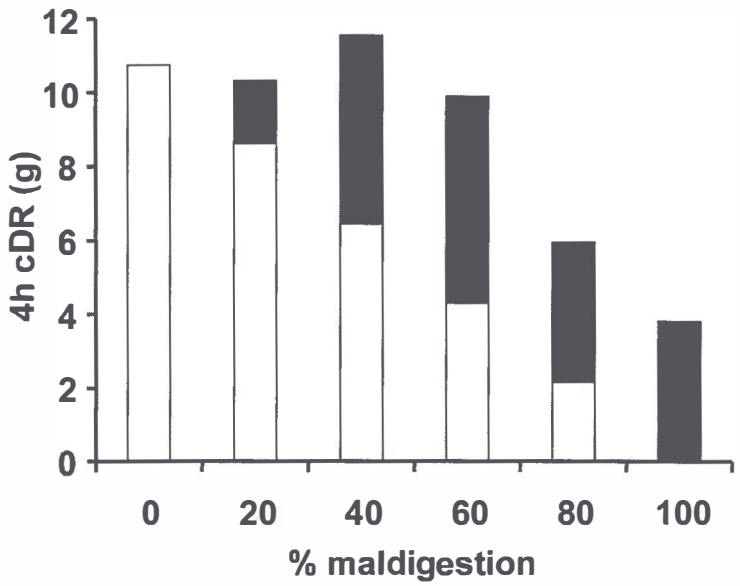

Figure 4: Effect on total 4h cDR of colonic $^{13}CO_2$ production at different rates of carbohydrate maldigestion.
White areas: metabolic generated $^{13}CO_2$ from consumed carbohydrate substrate.
Black areas: $^{13}CO_2$ production in the colon from fermented carbohydrate.
(For details of the model: see text)

Table II: 4h cDr in relation to consumed ^{13}C-lactulose dose

dose (g)	n	4h cPDR	s	x (g)	4h cDR	S
0 up to 9	7	16.6	16	10	1.7	1.6
10 up to19	22	25.7	15.9	20	5.1	3.2
20 up to 29	6	18.7	13.1	30	5.6	3.9
30 up to 39	5	9.4	7.7	40	3.8	3.1
40 up to 49	3	7.5	5.5	50	3.8	2.8

DISCUSSION:

Levitt[13] studied the relationship between H_2 concentration in the colon and in breath. He determined that 21% of the H_2 gas installed in the colon could subsequently be detected in breath. It was also concluded that this relation was almost constant and thus the H_2 concentration in breath represents the quantity of H_2 in the colon.

The H_2 breath test has been used to detect carbohydrate maldigestion since more than 2 decades[1-3]. However, after introduction into clinical practice it became clear that there were several factors that could influence the test result, making it less reliable. In the literature a highly variable number of "non- H_2 producers" is reported (up to 40%)[10] presumably partially dependent on experimental conditions[16].

Furthermore, hyperventilation, smoking or high fasting levels of H_2 can make interpretation of test results difficult and unreliable[17,18].

In an earlier publication we described the relation between both the H_2 and $^{13}CO_2$ exhalation in breath and the measured intestinal lactase activity after consumption of ^{13}C-lactose[14]. We found a discrepancy between both breath test results in 36% of cases. We concluded that in these situations with conflicting test results a biopsy should be performed to establish a diagnosis. To investigate the possible cause of these incongruous breath test results we studied gas production from carbohydrate in the colon, using ^{13}C-lactulose as a substrate. In this study we show that there was a variation in H_2 producer state at the same substrate dose in one of six individuals. In the other five subjects the producer state was unchanged during three consecutive tests. The variation in the response in one of the subjects illustrates the fact that the producer state of an individual can vary periodically and cannot always be determined simply by a single lactulose breath test. Furthermore, the variation in H_2 production in the same and in different individuals with the same test dose of lactulose shows that the H_2 breath test cannot be used for quantification of the degree of carbohydrate maldigestion in carbohydrate breath tests.

The $^{13}CO_2$ response in breath from colonic ^{13}C-lactulose fermentation also showed

a large intra- and inter-individual variation. No dose response relation could be seen.

The data demonstrate that $^{13}CO_2$ production in the colon can cause false-negative ^{13}C-carbohydrate breath test results, because the $^{13}CO_2$ originating from the colon is added to the exhaled $^{13}CO_2$ from digested and metabolized substrate. In particular at relatively low rates of maldigestion this effect can be relevant, as is shown in Fig. 4. The effect of colonic $^{13}CO_2$ production on the total $^{13}CO_2$ output in breath is especially great under the currently used standard test conditions (without significant physical activity). In these conditions the 4-h cPDR is low as a result of a low oxidation rate of absorbed substrate[14,19]. Changing the test conditions could possibly reduce this disturbing effect.

In a previous paper we reported higher sensitivity for the combined H_2 /$^{13}CO_2$ lactose breath test for detection of hypolactasia[14]. The positive correlation seen between H_2 and $^{13}CO_2$ excretion as observed after ingestion of ^{13}C-lactulose is presumably the underlying explanation. In low gas production states, in case of maldigestion the result of the H_2 breath test can be false negative. However, in those cases the chance of a false-negative $^{13}CO_2$ result is reduced owing to the associated low colonic $^{13}CO_2$ production. In high gas producing states, the $^{13}CO_2$ test has a relatively high probability of false-negative results caused by colonic $^{13}CO_2$ production. However, in those cases the H_2 results have a high probability of being truly positive in case of maldigestion. Our study with labelled lactulose confirms the relation between CO_2 and H_2 production in the colon found by Heresbach et al[20] using indirect calorimetry.

In conclusion: The interpretation of both H_2 and $^{13}CO_2$ breath tests for diagnosis of carbohydrate maldigestion can potentially be hampered by variation in colonic gas production. Although the colonic $^{13}CO_2$ production is highly variable, the response appears to be an important cause of false-negative results in particular at moderate rates of maldigestion. False negative H_2 and $^{13}CO_2$ breath test results generally do not coincide. The breath tests are therefore complementary in their diagnostic value.

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CHAPTER 5

Lactose (mal)digestion evaluated by the ^{13}C-lactose digestion test

Running head: Lactose (mal)digestion

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ABSTRACT

Background: The prevalence of genetically determined lactase non-persistence is based on the results of the lactose H_2 breath test. This test, however, is an indirect test, which might lead to misinterpretation.

Design: We determined lactase activity in healthy Chinese and Dutch students using a novel ^{13}C-lactose digestion test. The cut-off value of this test was established in a Chinese population with a homogenous genetic background of lactase non-persistence and was compared with the results obtained in a Caucasian population. Twenty-five grams of a ^{13}C-lactose solution was consumed by 12 known H_2-positive and 5 H_2-negative Chinese students and 48 Dutch students and, subsequently, ^{13}C-glucose concentration in plasma and H_2 excretion in breath were measured.

Results: A similar ^{13}C-glucose response curve was found in all Chinese students. The mean response curve in the Dutch students was more pronounced ($P<0.01$). The 1 h (peak) plasma ^{13}C-glucose concentration was the best discriminator between lactose digesting and maldigesting subjects. The cut-off level of 2 mmol/L^{-1} ^{13}C-glucose in plasma was defined in the H_2-positive Chinese students group. Based on the ^{13}C-glucose response, the prevalence of lactose maldigestion in the Dutch subjects was 25%; based on the lactose H_2 breath test 17%.

Conclusions: Using the ^{13}C-lactose digestion test, the results demonstrate a higher prevalence of lactose maldigestion in a Caucasian population than indicated by the results of the H_2 breath test. A moderate increase in the plasma ^{13}C-glucose concentration after consumption of ^{13}C-lactose in the young adult Chinese subjects indicates a residual lactase activity in that age group, even when a positive H_2 breath test result is obtained. These results indicate that the ^{13}C-glucose concentration in plasma more accurately reflects the small intestinal lactose digestion capacity than the lactose H_2 breath test.

INTRODUCTION

Prevalence of hypolactasia in different populations in the world has been well documented. Clear differences exist between Caucasians and individuals of non-Caucasian origin[1]. In many of these studies, a lactose H_2 breath test with unphysiological high doses of substrate (50 g, equivalent to the consumption of 1 liter of cows milk), is used. The shortcomings of the H_2 breath test are well known. The prevalence of non-H_2 producers according to Arola is 2-20%[2]. Moreover, antibiotics[3], low colonic pH[4] and other extra-intestinal factors (smoking, hyperventilation[5,6]) can interfere with the outcome of the test. False positive test results are nevertheless rare. In our recent clinical study in pediatric patients with various gastrointestinal diseases, the diagnosis of hypolactasia by measuring lactase activity in biopsy material and by performing the H_2 breath test were compared; false negative breath test results occurred in 46%[7].
In the present study, we used a more physiological dose of 25 g lactose and studied the H_2 breath response in 73 young healthy Chinese students. From this group with genetically determined lactase non persistence, a sample of individuals with a positive test result was used as a reference for lactose maldigestion. The results obtained from this group were compared with those of a group of Dutch students of comparable age with a known high prevalence of genetically determined lactase persistence and with those of the minority of Chinese subjects with a negative lactose H_2 breath test result.

SUBJECTS AND METHODS

Subjects

Seventy-three (35 male and 38 female, age range between 19 and 22 years) Chinese students at the West China University of Medical Sciences, Chengdu, China, and 48 (13 male and 35 female, aged 20 to 25 y) Dutch students at the University of Groningen, The Netherlands, were recruited for this study. All subjects were apparently healthy and had not been using antibiotics during the 10 days before the study. Verbal informed consent was obtained from all tested subjects. The study was approved by the ethical committee of both the West China University of Medical Sciences, Chengdu, China, and the University of Groningen, Groningen, The Netherlands.

Lactose

The unlabeled lactose was pure lactose (>99%) obtained from Spruyt Hillen (Utrecht, The Netherlands). The ^{13}C-lactose used in this study was obtained from the Dutch Institute of Dairy Research (NIZO), Ede, The Netherlands (Dr. R. van der Meer). This ^{13}C-lactose consisted of naturally ^{13}C-enriched lactose, derived from milk of cows fed with cattle fodder corn for 5 weeks. Milk of several cows was pooled. Lactose was obtained by standard procedures. The chemical purity of the ^{13}C-lactose powder was 95%. The ^{13}C abundance of ^{13}C-

lactose was 1.098% (δ $^{13}C_{PDB}$ -12 $^{o}/_{oo}$). Bacterial cultures of the ^{13}C-labeled and unlabeled lactose powder for enterococci, salmonellae, and other enteropathogens were negative.

Experimental protocol in Chengdu, China

All Chinese students and two Dutch students, who conducted this investigation in Chengdu, were subjected to this protocol. The data obtained from the two Dutch students were used as an internal control and were subsequently added to the data of the Dutch group.

Protocol breath hydrogen test

Subjects were advised to consume a low-fiber diet during the day before the experiment to lower the fasting breath H_2 concentration. After an overnight fast (>12 h), 25g of unlabelled lactose dissolved in 250 mL water was consumed by the subjects. The subjects remained quietly seated during the test period. They were allowed to drink water, coffee or tea (without sugar and cream) from two h after the lactose ingestion. No smoking was allowed, since smoking has been shown to increase breath H_2 concentration[6]. The end-expiratory air, which essentially represents alveolar air[8], was sampled by breathing through a straw into a 20-mL syringe. Breath air samples for H_2 determination were collected before and every 30 min after the lactose load for 6 hours.

Protocol ^{13}C-lactose test

The 5 Chinese subjects with an increase in breath H_2 concentration lower than 20 ppm in this 6-hour experiment (possibly non-producers) were further evaluated by using ^{13}C-lactose as substrate. Additionally twelve of the 68 Chinese students with a breath H_2 concentration higher than 20 p.p.m. after consumption of 25 g of lactose were also recruited for the ^{13}C-lactose test (randomly selected). The subjects were asked to refrain from consuming foods naturally enriched in ^{13}C, such as cane sugar, corn, corn products and pineapple, for two days before the experiment. The substrate, 25 g ^{13}C-lactose in 250 mL water, was administered orally. A venous catheter (Becton Dickinson GMBH, Heidelberg, Germany) was placed in an antecubital vein which allowed repetitive blood sampling. Samples of 2 mL of venous blood were taken 30 minutes before and 0, 60, 120, 180, 240, 300 and 360 min after substrate ingestion. The blood-sampling tubes (Vacutainer®, Becton Dickinson GMBH, Heidelberg, Germany) contained NaF and potassium oxalate. The other experimental conditions were similar to those as described above for the initially conducted breath hydrogen test.

Experimental protocol in Groningen, The Netherlands

All 48 Dutch students consumed 25 g ^{13}C-lactose in 250 mL of water, while

simultaneously performing the H_2 breath test and the ^{13}C-lactose digestion test. The experimental conditions were identical to those conducted in Chengdu.

Analytical methods

1. Breath H_2 determination: a H_2 monitor (type 60HP, Stimotron Medizinische Geraete, Wendelstein, Germany) was used to measure the breath hydrogen concentrations. The monitor was calibrated using a standard gas containing 96 ppm H_2. To evaluate the validity of the monitor, some breath air samples were both determined by the monitor and gas chromatography, which is routinely used in the Groningen laboratory[9]. The results showed that H_2-positive and -negative persons evaluated by H_2 monitor and by gas chromatography were identical.

2. Plasma ^{13}C-glucose determination: one aliquot of serum was analyzed for glucose concentration applying routine techniques by using an ECA-180 glucose analyzer (Medingen, Germany), and a second aliquot was prepared for $^{13}C/^{12}C$ analysis. This method was first described by Normand *et al*[10]; we used the same method with some modifications. 100 μL plasma was added to 1 mL ethanol for denaturation of plasma proteins. After vortex mixing and centrifugation the supernatant was transferred to 2 mL vials. After evaporation to dryness, sugars were derivatized to the penta-acetate derivative using 75 μL acetic acid anhydride / pyridine (10:5 v/v) reacting for 1.5 h at room temperature. After evaporation of the reagent, the derivatives were dissolved in 500 μL chloroform.

The $^{13}C/^{12}C$ ratio of glucose was determined applying Gas Chromatography/ Combustion/Isotope Ratio Mass Spectrometry (GC/C/IRMS). A Delta S/GC instrument (Finnigan MAT, Bremen, Germany) was used. The GC conditions were as follows: 2 μL of the chloroform solution were injected in the splitless mode onto a 25m x 0.32 mm (0.2 μm film thickness) OV1701 column (CP Sil 19CB, Chrompack, Middelburg, The Netherlands) installed in a Varian 3300 GC. The oven temperature was programmed from 100°C (1 min) to 275°C (2 min) at a rate of 30^0C min^{-1}. Helium was used as the carrier gas at a column head pressure of 20 psi. Eluting compounds were combusted on-line in a platin catalyzed CuO oxidation reactor operating at 800 °C. Solvent and compounds eluting before 4 min were kept out of the reactor applying a helium backflush gas flow. Water vapor was removed by a nafion tubing and the CO_2 pulses formed in the reactor are transferred to the IRMS through an open split interface. $^{13}CO_2$ /$^{12}CO_2$ measured by IRMS is corrected for ^{17}O abundance[11] and the final ^{13}C /^{12}C ratio is expressed as $\delta\ ^{13}C_{PDB}$[12].

Calculations

The $\delta\ ^{13}C_{PDB}$ value is converted to the atom % value (AP). The AP values after

ingestion of substrate are corrected for the baseline abundance. The difference (atom % excess (APE)) is used for further calculations. For breath CO_2, the APE value is related to the standard CO_2 excretion (300 mmol/m² BSA h^{-1}) in order to calculate the substrate derived exhalation rate. This exhalation rate is calculated as % dose recovered (PDR h^{-1}). Total recovery after 6 h is calculated as the cumulative % dose (cPDR 6h). The $^{13}C/^{12}C$ of plasma glucose is converted to the AP ^{13}C. The concentration of glucose derived from the ^{13}C-lactose (exogenous glucose) in plasma is calculated as follows:

$$[^{13}C\text{-glucose}] = [\text{total glucose}] \times ((AP\ ^{13}C_t - AP\ ^{13}C_{t=0})/(AP_{substrate} - AP_{body}) \times 2.67$$

in which:

[total glucose]	=	glucose concentration in plasma (mmol L^{-1})
AP $^{13}C_t$	=	atom % ^{13}C of plasma glucose at time point *t* after ingestion of ^{13}C lactose
AP $^{13}C_{t=0}$	=	atom % ^{13}C of plasma glucose before ingestion of ^{13}C lactose
AP $^{13}C_{substrate}$	=	atom % ^{13}C of the administered lactose
AP $^{13}C_{body}$	=	atom % ^{13}C of endogenous glucose, expressed by the basal ^{13}C abundance in breath
2.67	=	factor to correct for the dilution of glucose ^{13}C abundance the ^{13}C abundance of the derivatizing acetate C atoms

Data analysis and Statistics

The mean H_2 concentration (p.p.m.) of two breath samples before lactose ingestion was taken as the basal value. Subsequent ΔH_2 concentrations were expressed as the increment above this basal level. When the increment exceeded 20 p.p.m. the subject was considered to be a lactose maldigester (H_2-positive). This criterion was used in the non-labeled lactose pilot study as well in the ^{13}C-lactose study.

The baseline value of plasma glucose ^{13}C-abundance was calculated from two samples collected before ^{13}C-lactose ingestion. Plasma glucose concentration enrichment was defined as the increment of ^{13}C above the baseline ^{13}C-abundance level.

Unpaired Student's *t*-test (two tailed) was used to test the differences of mean values when two (sub)groups were assessed. $P<0.05$ was considered to be significant.

RESULTS

To evaluate the role of the ^{13}C-lactose digestion test for the diagnosis of hypolactasia, this test was first applied in healthy Chinese students presumed to be lactose maldigesters. First a screening was made in 73 Chinese students with 25 g unlabeled lactose: 93% of these subjects were H_2-positive and 7%

H_2-negative. From the H_2-positive Chinese students (considered to be true maldigesters), 12 were randomly included in consecutive studies with ^{13}C-lactose together with all 5 Chinese H_2-negative students. In all subjects, the serum ^{13}C-glucose concentration rise and H_2 response were measured after substrate ingestion. In the 12 initially H_2-positive individuals, the H_2 response result was identical in the repeated test, in one of 5 screened negative individuals the H_2 response became positive in the repeated test. The results of this individual are not included because of the fact that our suspicion of false negative H_2 result in the first test could not be proven retrospectively. Fig.1 shows the mean total glucose response and the mean fractional ^{13}C-glucose response in plasma in the 12 H_2-positive Chinese students. The mean total glucose concentration did not show an increase after lactose digestion. However, a clear increase in ^{13}C-glucose concentration in plasma was found, representing ^{13}C-substrate derived serum glucose, which was not expected in these adult individuals with genetically determined low lactase activity. A maximal ^{13}C-glucose concentration increase

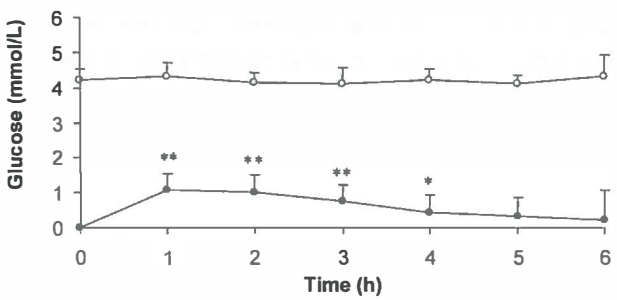

Figure 1. The (total) glucose concentration (lactose digestion test, LDT) (O-O) and the ^{13}C-glucose concentration (^{13}C-LDT) (●-●) in plasma of 12 H_2-positive Chinese students after consumption of 25 g ^{13}C-lactose. Mean values ± SD. *: P<0.05; **: P<0.01.

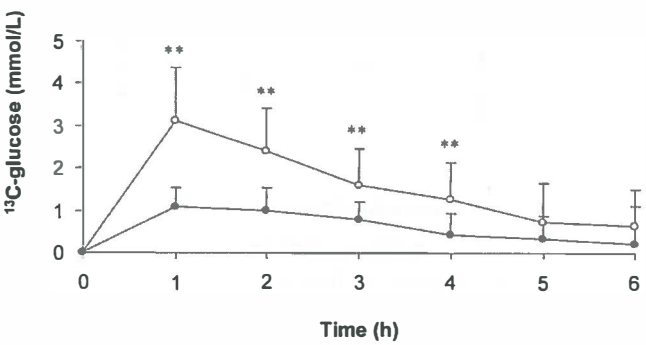

Figure 2. ^{13}C-glucose concentration in plasma of H_2-positive Chinese students (n=12) (malabsorbers; ●-●) and of H_2-negative Dutch students (n=40) (absorbers; O-O) after consumption of 25 g ^{13}C-lactose. Mean values ± SD. **: P<0.01.

was found 1 h after substrate ingestion. In Fig. 2 the ^{13}C-glucose response in these H_2-positive Chinese students is compared with the response in the 40/48 H_2-negative Dutch students, assumed to be lactose digesters because of their genetical background. The difference was maximal at 1 h after lactose consumption ($P<0.01$). Calculating the area under the curve of ^{13}C-glucose showed no improvement in discriminating digesting and maldigesting subjects (data not shown). Therefore, the 1 h-value of plasma ^{13}C-glucose concentration was used as criterion for evaluation of hypolactasia. The students of Chengdu, a population which can be considered as relatively genetically homogenous, were considered as a reference group for lactase non-persistence. From this group, 12 H_2-positive individuals were randomly selected. The 1 h ^{13}C-glucose cut-off value (mean value of the group plus 2 SD) turned out to be 2.0 mmol L^{-1}. With this cut-off value the H_2-negative Chinese students (considered as possibly false negative maldigesters, due to their genetical background) and a population of 48 Dutch students were analyzed. From the Dutch group, 17% were H_2-positive and 83% H_2-negative after consumption of 25 g lactose. In Fig. 3 the results of the ^{13}C-lactose digestion test are shown. All H_2-positive Chinese students had ^{13}C-glucose concentrations below 2 (by definition); all but

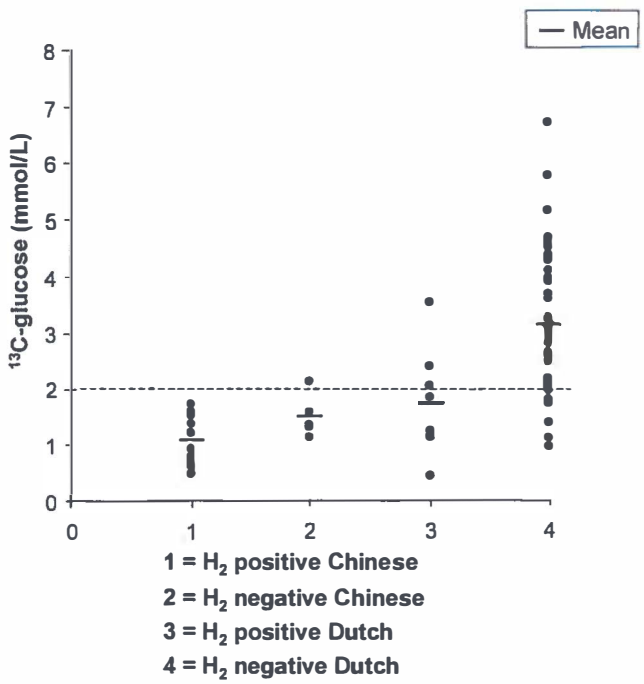

Figure 3. 1 h ^{13}C-glucose values in plasma after consumption of 25 g ^{13}C-lactose. Four groups were compared: 1: H_2-positive Chinese students (n=12), 2: H_2-negative Chinese students (n=4), 3: H_2-positive Dutch students (n=8) and 4: H_2-negative Dutch students (n=40).

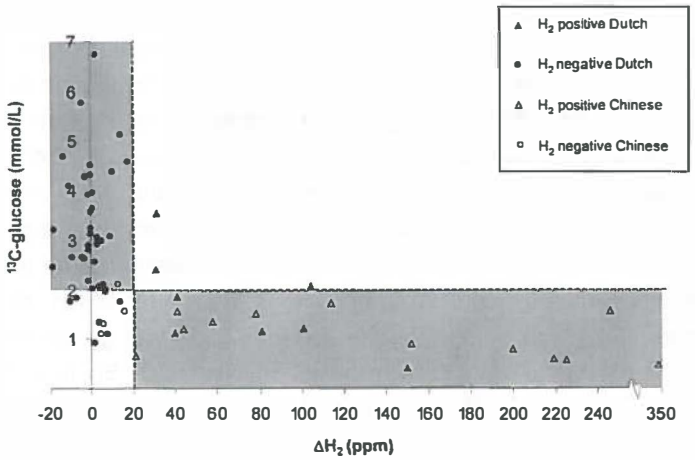

Figure 4. 1 h ^{13}C-glucose values in plasma plotted against ΔH_2 values in breath of H_2-positive Chinese (n=12) (▲), H_2-negative Chinese (n=4) (○), H_2-positive Dutch (n=8) (▲) and H_2-negative Dutch (n=40) (●) students after consumption of 25 g ^{13}C-lactose.

one of the H_2-negative Chinese students had values below the cut-off value. From the 8 Dutch H_2-positive students, 5 exhibited ^{13}C-glucose values below the cut-off value for ^{13}C-glucose. From the 40 Dutch H_2-negative group, 7 had low ^{13}C-glucose values.

To analyze the discrepancy in more detail, ^{13}C-glucose values from all 48 Dutch students and 16 Chinese students were plotted against their increase in H_2 values (fig. 4). In the disconcordant area (indicated as blank area) a considerable number of H_2-negative Dutch students were found with low ^{13}C-glucose concentrations. Two of the three H_2-positive students with high ^{13}C-glucose concentrations had a late H_2-response (increase in H_2 values after 3 h instead of 2 h) possibly indicating non lactose related colonic fermentation processes. Using the combined H_2 and ^{13}C-glucose values (at least one of both tests positive), we extrapolated that 99% and 25% of the Chinese and Dutch population, respectively, had lactase non-persistence.

DISCUSSION

Adequate diagnosis of hypolactasia or lactase non-persistence is relevant for clinical and epidemiological studies. The existing techniques of the lactose tolerance test (LTT), H_2 breath test and small intestinal lactase enzyme activity assay have well-known shortcomings[2]. Therefore, we developed a ^{13}C-lactose digestion test. This test allows for the discrimination between the origin of exogenous (substrate derived) plasma glucose and endogenous glucose. To prove the diagnostic power and establish the cut-off values, we applied this technique first in a genetically homogenous population in Chengdu, China, with a very high (presumably 100%) prevalence of genetically determined low

intestinal lactase activity. The individuals with a positive lactose H_2 breath test result in this population were considered as the reference group of lactose maldigestion, as it is known that this test has an almost negligible proportion of false positive results. To our surprise, a considerable rise in ^{13}C-glucose concentration in plasma was observed after ^{13}C-lactose consumption. Obviously, this adult population still has some lactase activity available, which allows lactose digestion and absorption of the hydrolysis products (glucose and galactose) to a certain extent. Despite this observation, we still consider this group as the best available reference group of lactose maldigestion. A mutation, which caused the phenotype of lactase persistence into adulthood, was genetically selected especially in cattle breeding communities in Northern European regions[1]. Therefore our homogenous Chinese reference group, without known selection of the lactase persistence mutation, is preferable over any heterogeneous patient group with symptoms and biopsy proven low lactase activity. In such a patient group the overall lactose digesting capacity is difficult to establish reliably and cannot simply be derived from measuring lactase activity in one single biopsy sample.

The largest difference in ^{13}C-glucose response between lactose digesting and maldigesting individuals was found 1 h after consumption of labeled lactose. Also, theoretically, one can expect that with a time period < 1 h variations in gastric emptying will influence the outcome, and with a time period > 2 h variations in hepatic glucose metabolism will play a more prominent role. The 1 h ^{13}C-glucose rise was therefore used as parameter to discriminate between both groups, with a cut-off level of 2 mmol L^{-1}. The H_2-negative Chinese students had a similar ^{13}C-glucose concentration compared to their H_2-positive group members , which indicates that all but one were false negative in the H_2-response. When we combine the results of the initially positive Chinese subjects in the unlabeled lactose H_2-screening test (confirmed by the ^{13}C-labeled lactose test) and the serum ^{13}C-glucose concentration rises in the subsequent ^{13}C-lactose digestion test, a prevalence of hypolactasia in the Chinese population of 99% is extrapolated. This finding confirms the expected fact that in lactose maldigesting subjects, false negative H_2 breath test results occur.

Using the same criteria for the Dutch students, we also found a considerable number of false negative H_2 responders. With the H_2 test a prevalence of 17% lactose maldigestion was found, which is higher than found in previous studies in Dutch population samples (2% (children) – 8% (adults with gastrointestinal complaints))[1]. Using the new ^{13}C-lactose digestion test, a prevalence of 25% was found. It is clear that additional studies with larger numbers of subjects have to be performed to obtain an accurate value for the prevalence in the whole Northern European population. Our novel test could possibly be performed by a two sample venous micro puncture technique at time zero and one hour after substrate ingestion.

Acknowledgements:

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CHAPTER 6

The $^{13}C/^{2}H$-glucose test for determination of small intestinal lactase activity

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ABSTRACT

Background: To diagnose hypolactasia, determination of lactase enzyme activity in small intestinal biopsy material is considered to be the golden standard. Because of its strongly invasive character and the sampling problems, alternative methods have been looked for.

Design: We analyzed the ^{13}C-glucose response in serum after consumption of 25 g of naturally enriched ^{13}C-lactose. As internal standard 0.5 g of ^{2}H-glucose was added and the ^{2}H-glucose response in serum was measured simultaneously. The studies were performed in healthy volunteers with a background of genetically determined lactase non-persistence (n =12; low lactase activity) and lactase persistence (n =27;high lactase activity). The results were compared with those of the lactose hydrogen breath test, the lactose $^{13}CO_2$ breath test and the previously described ^{13}C-lactose digestion test.

Results: After consumption of ^{13}C-lactose and ^{2}H-glucose the mean ratio ^{13}C-glucose/^{2}H-glucose concentration in serum at 45 – 75 min was 0.26± 0.09 in the low lactase activity group and 0.93± 0.17 in the high lactase activity group (P<0.01). Threshold of the ratio between digesters and maldigesters was calculated as 0.46. Accuracy of the new test was superior to all other tests.

Conclusions: We conclude that the ^{13}C/^{2}H-glucose test has the potential of determining the small intestinal lactase activity *in vivo* and of estimating the amount of lactose which is digested in the small intestine.

INTRODUCTION.

Data about global prevalence of lactase non-persistence are mainly based on the outcome of the lactose-hydrogen breath test[1-7]. This test, however, might result in a false negative outcome, leading to underestimation of the prevalence data. The commonly-accepted golden standard of determining hypolactasia is the analysis of the lactase enzyme activity in small intestinal biopsy material. Because of its strongly invasive character, alternative methods have been developed. In a previous study, we analyzed the ^{13}C-glucose response after consumption of 25 g naturally enriched ^{13}C-lactose[8]. We applied this method in a study of adults with a background of genetically determined lactase non-persistence and observed a clear increase in serum concentration of lactose derived glucose after consumption of 25 g of lactose, indicating unexpected high residual lactase activity. In the present study, we improved this test by adding simultaneously an internal reference sugar (^{2}H-glucose) to compensate for interfering factors as gastric emptying, small intestinal transit time and insulin effects. The measured response, the ratio ^{13}C-glucose/^{2}H-glucose concentration in serum, is considered to be the reflection of the *in vivo* lactase activity and represents a quantitative and physiological indicator for the overall lactose digestion capacity.

SUBJECTS AND METHODS

Subjects.

12 students (5 male, 7 female; mean age 25.9 $\pm$ 4.4 years) from China (2), Vietnam (3), Tanzania (2), Nigeria (1), Eritrea(1), Egypt (1), Japan (1) and Afghanistan (1), with a genetically determined lactase non-persistence and 27 Dutch students (8 male, 19 female; mean age 23.6 $\pm$ 3.9 years) with a genetically determined lactase persistence, all from the University of Groningen, The Netherlands, were recruited for this study. All subjects appeared to be healthy and had not been using antibiotics or other drugs affecting intestinal function during the 10 days preceding the study. Verbal informed consent was obtained from all tested subjects. The study was approved by the ethical committee of the University of Groningen.

Substrates.

The ^{13}C-lactose used in this study was obtained from the Dutch Institute of Dairy Research (NIZO), Ede, The Netherlands (Dr. R. van der Meer). This ^{13}C-lactose consisted of naturally ^{13}C-enriched lactose, derived from milk of cows fed with cattle fodder corn for 5 weeks. Milk of several cows was pooled. Lactose was obtained by standard procedures. The chemical purity of the ^{13}C-lactose powder was > 95%. The ^{13}C abundance of ^{13}C-lactose was 1.096% ($\delta\ ^{13}C_{PDB}$ – 13.7 $^{o}/_{oo}$). Bacterial cultures of the ^{13}C-labeled lactose powder for enterococci, salmonellae, and other enteropathogens were negative. ^{13}C-glucose (dextrose,

Natuproducts BV, Harderwijk, The Netherlands) had a ^{13}C-abundance of 1.097% ($\delta\ ^{13}C_{PDB}$ –12.7 $^{o}/_{oo}$). 6,6-^{2}H-glucose, 98% ^{2}H was obtained from Isotec Inc (Miamisburg, Ohia, USA). Isotopic purity was confirmed by GC/MS.

EXPERIMENTAL PROTOCOL.

The subjects were asked to refrain from consuming foods naturally enriched in ^{13}C, such as cane sugar, corn, corn products and pineapple, for the 3 days preceding the experiment. The substrates, 25 g ^{13}C-lactose or 26.3 g ^{13}C-glucose together with 0.5 g ^{2}H-glucose dissolved in 250 ml water, were administered orally. A venous catheter (Becton Dickinson GMBH, Heidelberg, Germany) was placed in an antecubital vein which allowed repetitive blood sampling. 2 mL. samples of venous blood were taken 30 and 15 min before substrate consumption. The first 2 h thereafter, samples were collected every 15 min and during the following 4 h every 30 min.The blood-sampling tubes (Vacutainer®, Becton Dickinson GMBH, Heidelberg, Germany) contained sodium fluoride and potassium oxalate.

Breath samples for analyzing H_2 and $^{13}CO_2$ were taken twice before ingestion of ^{13}C-lactose and every 15 min during the first 2 h and every 30 min during the following 4 h after ingestion.

Analytical methods.

Plasma ^{13}C-glucose and ^{2}H-glucose analysis.

One aliquot plasma was used for routine analysis of the glucose concentration, applying an ECA-180 glucose analyzer (Medingen, Dresden, Germany). A second aliquot (100 μL) was prepared for the measurement of the ^{13}C and ^{2}H enrichment. The sample preparation procedure was as described in a recent study[8,9]. The $^{13}C/^{12}C$ isotope ratio measurement of the glucose penta-acetate derivative was determined by Gas Chromatography / Combustion / Isotope Ratio Mass Spectrometry (GC/C/IRMS) as previously described[8,9]. For the translation of ^{13}C abundance measured for the C-atoms of the derivatized molecule, the method was modified. Originally a constant value of 2.67 was used to convert derivative carbon enrichment into glucose carbon enrichment, whereas the baseline glucose carbon ^{13}C abundance was reflected by the baseline $^{13}CO_2$ abundance in breath. In this study, the absolute ^{13}C abundance of the glucose carbons was calculated as follows. With each series of plasma samples two reference glucose samples were included: one corn glucose and one potato glucose with known ^{13}C abundance determined by total combustion IRMS. By comparison of the measured ^{13}C abundance of the derivatized molecules with that of the combustion values of the original glucose, the ^{13}C abundance of the acetate carbon atoms could be calculated for each reference sample. Using this value the ^{13}C abundances of the derivatized plasma glucose were transferred to the ^{13}C abundance of the plasma glucose carbon.

The ^{2}H enrichment was measured by Gas Chromatography / Mass Spectrometry (GC/MS). For this purpose the glucose penta-acetate derivative was separated on a 20 m x 0.18 mm (0.4 μm film thickness) AT 1701 capillary column (Alltech Ass. Inc., Deerfield, Illinois, USA) applying the following GC conditions. The initial column temperature was 80 °C for 1 min. and was increased to 280 °C at a rate of $30^{0}C\ min^{-1}$ The sample (2 μL) was injected in the splitless mode at an injector temperature of 275 °C. Helium served as the carrier gas at a constant flow rate of 0.7 mL min^{-1}. The capillary GC column was connected with a low dead volume connector to a permanent uncoated fused silica capillary inserted into the ion source of the mass spectrometer. The interface temperature was 280 °C. The GC/MS system used was a Finnigan SSQ7000 quadrupole instrument including a HP 5980 GC with electronic flow controlling facilities. The MS was used in the electron impact mode and selected ion monitoring was performed for the mass ions m/z 242 (M0) and 244 (M2). The acquisition time for each ion was 100 msec assuring at least 20 data points over the GC peak. The signal intensity level was adjusted to fit a known plateau level assuring reproducible and correct M2/M0 isotope area ratios. The baseline M2/M0 area ratio, determined on one day at 28 samples of 14 healthy volunteers, was 2.2 ± 0.06 %, whereas the theoretical value is 1.9 %. Linearity of the M2/M0 area ratio in the range of 0 – 10 mol% ^{2}H-glucose was good ($r > 0.99$).

Plasma insulin analysis.

A third aliquot (150 μl) was used to measure plasma insulin concentrations (in duplicate) by using a radioimmunoassay kit produced by LINCO Research, Inc., St. Charles, MO, USA.

Breath H_2 determination.

Breath H_2 analysis was performed with the gas chromatography technique, which is routinely used in the Groningen laboratory[10]. The mean H_2 concentration (parts per million; ppm) of two breath samples before lactose ingestion was taken as the basal value. ΔH_2 concentrations were expressed as the increment above the basal level. When an increment in excess of 20 ppm was sustained, the subject was considered to be H_2-positive.

Breath $^{13}CO_2$ determination

The analysis of $^{13}CO_2$ in breath was performed according to Koetse et al[11]. The ^{13}C-abundance of CO_2 was determined by continuous flow isotope ratio mass spectrometry (BreathMAT, Finnigan MAT, Bremen, Germany).

Calculations.

The ^{13}C-glucose concentrations were calculated as follows:

[^{13}C-glucose] = [total glucose] x (AP^{13}C-glucose$_t$ – AP^{13}C-glucose$_{t=0}$)/ (AP^{13}C-substrate -AP^{13}C-glucose$_{t=0}$).
[^{13}C-glucose]/ dose of ^{13}C-lactose expresses the [^{13}C-glucose] as % dose L^{-1}
AP^{13}C-acetate = (16xAP^{13}C-derivatized reference glucose – 6xAP^{13}C-reference glucose)/10.
AP^{13}C-glucose = (16xAP^{13}C-derivative – 10xAP^{13}C-acetate)/6.

in which:

- AP^{13}C-derivative = atom percentage ^{13}C of the derivatized plasma glucose.
- AP^{13}C-glucose$_t$ = atom percentage ^{13}C of plasma glucose carbon at time point (t) after ingestion of ^{13}C-lactose after conversion of the atom percentage ^{13}C of the derivative carbon;
- AP^{13}C-glucose$_{t0}$ = atom percentage ^{13}C of plasma glucose carbon before ingestion of ^{13}C- lactose after conversion of the atom percentage ^{13}C of the derivative carbon;
- AP^{13}C-substrate = atom percentage ^{13}C of the administered lactose determined by total combustion IRMS.
- 10 and 6 = the number of acetate carbon atoms and glucose carbon atoms respectively.

The ^{2}H-glucose concentrations were calculated as follows:
The M2/M0 area ratios determined by GC/MS were converted to molar ratios applying the calibration curve. The molar fraction ^{2}H-glucose was multiplied by the total glucose concentration in mmol L^{-1} to give the ^{2}H-glucose concentration, which was then expressed as a percentage of the administered dose: % dose L^{-1} .The ratio [^{13}C-glucose]/ [^{2}H-glucose] is determined based on the values in % dose L^{-1}.

Statistics
Unpaired Student's *t*-test (two-tailed) was used to test the differences of mean values when two (sub)groups were assessed. $P<0.05$ was considered to be significant.

RESULTS

The lactose digestion was studied in a group of healthy volunteers with expected lactase non-persistence (n =12) and expected lactase persistence (n =27). In the group with expected lactase non-persistence, those with a positive H_2-response after ingestion of 25 g of lactose were defined as lactose maldigester (n =10). In the group with expected lactase persistence, those with a negative H_2-response after lactose consumption were selected and considered as lactose

digesters (n =25). As is shown in Table 1, the resulting four groups can now be evaluated. The ^{13}C-lactose was consumed by all groups. The test results as shown in Fig.1 are in agreement with the results obtained before in a Chinese population[8]. Comparing the ^{13}C-glucose concentrations (mmol L^{-1}) over the first part of the experiment, a clear difference in ^{13}C-glucose response between the digester group and the maldigester group was found ($P < 0.001$; t = 15-120 min). In the same groups the kinetics of the reference sugar, ^{2}H-glucose, were also studied. In both groups, a clear response of this reference sugar was demonstrated (Fig. 1). In Fig. 2 the kinetics of this reference sugar ^{2}H-glucose are compared in the digester and maldigester group. The maximum in ^{2}H-glucose concentration in the digester group is lower than in the maldigester group. In order to study possible underlying differences in glucose kinetics between both groups, we repeated the experiment in a subgroup (four maldigesters, seven digesters), replacing ^{13}C-lactose by an equivalent dose of ^{13}C-glucose. In Fig. 3 the results of this control study are shown. No significant differences in ^{13}C-

Table 1: Definition of groups

	Volunteers with expected lactase nonpersistence (n =12)	Volunteers with expected lactase persistence (n = 27)
H_2-positive	10 (lactose "maldigesters")	2
H_2-negative	2	25 (lactose "digesters")

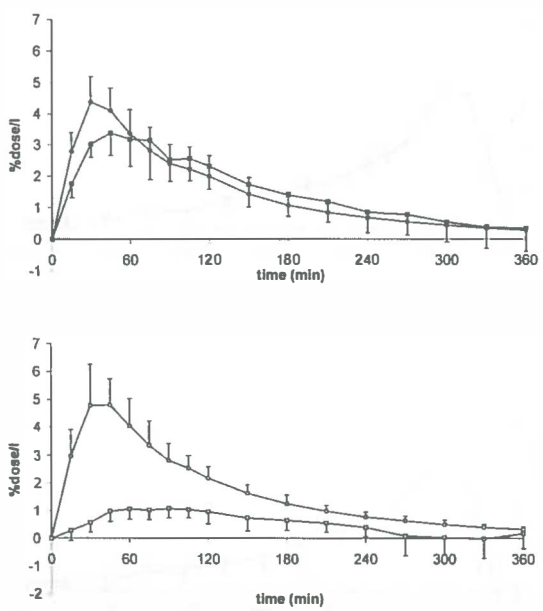


Figure 1. The concentration of ^{13}C-glucose (□-□, ■-■) and ^{2}H-glucose (○-○, ●-●) in serum after consumption of 25 g ^{13}C-lactose and 0.5 g ^{2}H-glucose in healthy volunteers defined as lactose maldigesters (□-□, ○-○; n = 10) and lactose digesters (■-■, ●-●; n = 10). Mean values ± SD.

glucose response were found between the digester and maldigester group. The ^{2}H-glucose response was also identical in both groups. Therefore, the difference in ^{2}H-glucose response between the digester and maldigester group after ^{13}C-lactose ingestion (Fig. 2) is most probably caused by differences in intestinal influx of ^{13}C-glucose and the resulting variation in the insulin response. This is clearly demonstrated in Fig. 4; the insulin response is low in the maldigester group after administration of ^{13}C-lactose, but is normal after administration of ^{13}C-glucose. Fig. 5(a) shows the ratio of the ^{13}C-glucose concentration and the ^{2}H-glucose concentration in serum after consumption of a mixture of ^{13}C-lactose and ^{2}H-glucose. As is shown in this figure, this ratio is increasing in both groups

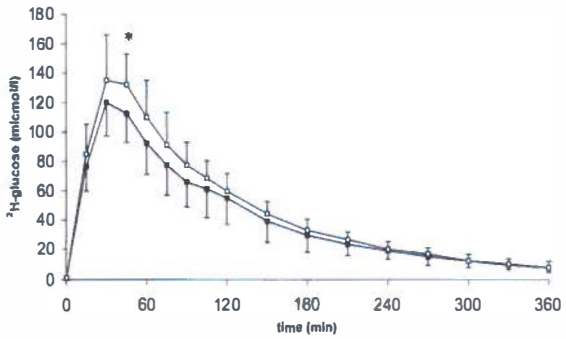
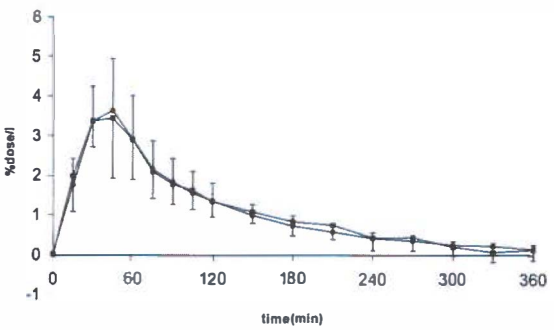
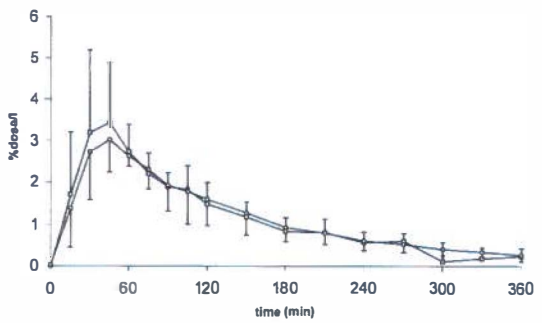


Figure 2. The concentration of ^{2}H-glucose in serum after consumption of 0.5 g ^{2}H-glucose and 25 g ^{13}C-lactose in healthy volunteers defined as lactose maldigesters (❑-❑; n = 10) and lactose digesters (■-■; n = 10). Mean values ± SD. * $P < 0.05$.

Figure 3. The concentration of ^{13}C-glucose (❑-❑, ■-■) and ^{2}H-glucose (○-○, ●-●) in serum after consumption of 26.3 g ^{13}C-glucose and 0.5 g ^{2}H-glucose in healthy volunteers defined as lactose maldigesters (❑-❑, ○-○; n = 4) and lactose digesters (■-■, ●-●; n = 7). Mean values ± SD.





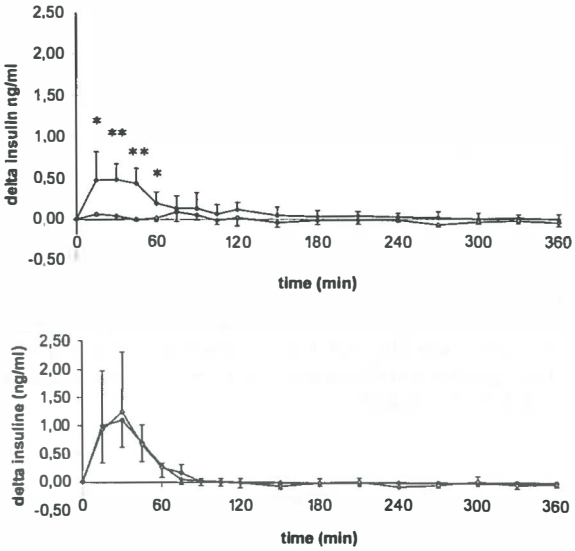


Figure 4. Insulin response in serum after consumption of (a) 25 g ^{13}C-lactose and 0.5 g ^{2}H-glucose and (b) 26.3 g ^{13}C-glucose and 0.5 g ^{2}H-glucose, in healthy volunteers defined as lactose maldigesters (□-□; ○-○ n = 4) and lactose digesters (■-■; ●-● n = 7). Mean values ± SD. * $P < 0.05$; ** $P < 0.01$.

during the first 90 minutes. In the maldigester group, the ratio does not exceed 0.4 and in the digester group it increases from 0.6 to about 0.8 during the first 90 minutes. To derive an index from this test, the mean ratio of 3 time samplings (45, 60 and 75 min) was calculated. In the maldigester group this index was 0.26 ± 0.10. As cut-off value this mean + 2 SD = 0.46 will be used. In the digester group the value for the index was 0.93 ± 0.17. The observed differences in the ratio of the digester and maldigester group are considered to be due to differences in small intestinal lactase activity. The same index was calculated using ^{13}C-glucose as a substrate instead of ^{13}C-lactose. Figure 5(b) shows that the index was 0.98 ± 0.08 and 1.03 ± 0.10 in the digester and maldigester group, respectively.

To establish whether addition of the reference sugar ^{2}H-glucose to the substrate improved the discriminative power for detection of hypolactasia, a comparison is made between the outcome of the previously described ^{13}C-lactose digestion test and the outcome of the $^{13}C/^{2}H$-glucose test. In the four groups as defined in Table 1, both tests were applied and the results are shown in Fig. 6. The discriminative power is clearly increased by adding the reference sugar. Figure 7 illustrates typical examples in applying the test, in which the importance of addition of a reference sugar is also underlined. Maldigestion in the middle panel is due to genetically determined lactase non-persistence, while maldigestion in the lower panel is most probably due to a systemic sugar transport defect due to vasovagal collapse of the lactase-persistent student. In Table 2 a comparison is made between four tests that can be used to diagnose hypolactasia. As shown, the accuracy of the $^{13}C/^{2}H$-glucose test is superior to the three other tests.

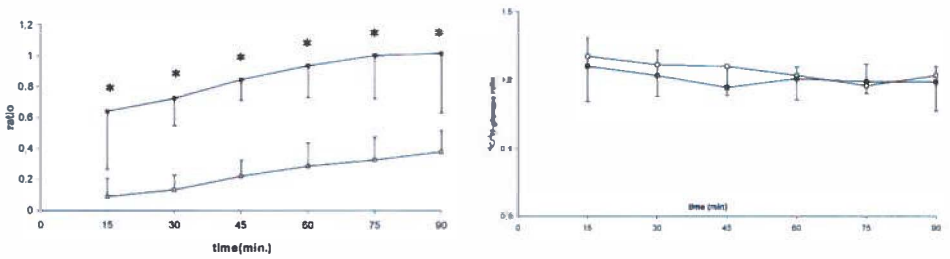


Figure 5. The ratio ^{13}C-glucose/ ^{2}H-glucose in serum after consumption of (a) 25 g ^{13}C-lactose and 0.5 g ^{2}H-glucose in healthy volunteers defined as lactose maldigesters (❑-❑, n = 10) and lactose digesters (■-■, n = 25); and (b): 26.3 g ^{13}C-glucose and 0.5 g ^{2}H-glucose in healthy volunteers defined as lactose maldigesters (❑-❑; n = 4) and lactose digesters (■-■; n = 7). Mean values ± SD. * P < 0.001.

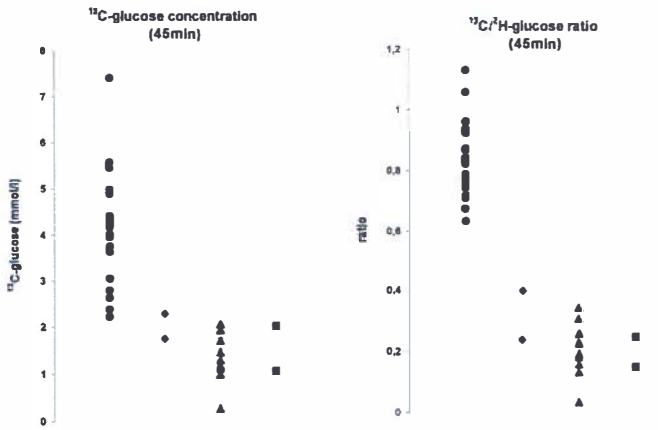


Figure 6. Comparison of the ^{13}C-glucose concentration (45 min) and the ratio of ^{13}C-glucose/^{2}H-glucose concentration (45 min) in healthy volunteers with an expected lactase persistence (●a+◆b) and an expected lactase non-persistence (▲a+■b). a, H_2 neg; b, H_2 pos.

DISCUSSION.

In a previous study, in order to develop an *in vivo* lactase activity test, we applied the ^{13}C-lactose digestion test in a population of Chinese medical students. We used ^{13}C-lactose as a substrate in this reference population with a genetically determined lactase non-persistence and a positive H_2-breath test. In these "lactase non-persistent persons" we observed a residual intestinal capacity for hydrolyzing lactose. This capacity was significantly lower than in the lactase-persistent persons, which was concluded from a comparison between the results of Chinese and Dutch students. In the present study, to exclude other causes for the measured differences between these groups at another level than the lactase enzyme activity (for instance variations in gastric emptying, small intestinal transit time and insulin effects), the handling of the reference sugar

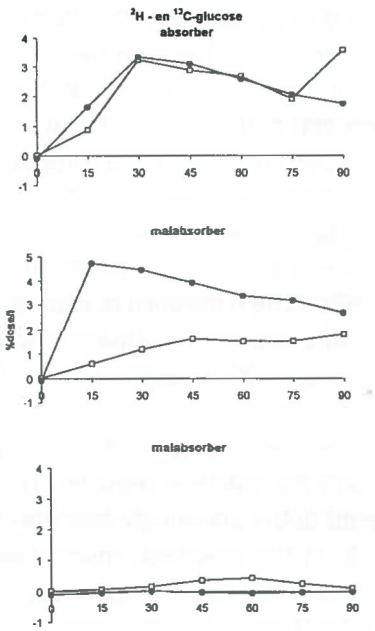


Figure 7. Typical examples of ^{13}C-glucose and ^{2}H-glucose response in (a) digester, (b) maldigester, due to genetically determined lactase non-persistence, and (c) maldigester, due to general intestinal malabsorption (●: ^{13}C-glucose, and ❑: ^{2}H-glucose).

Table 2. Comparison of diagnostic methods.

Test / parameter	Accuracy in group with expected lactase non-persistence
H_2 lactose breath test: cut-off value: H_2 > 20 ppm	10
$^{13}CO_2$ lactose breath test: cut-off value: $^{13}CO_2$ (4 h C_{PDR}) < 14.5%	3
^{13}C-lactose digestion test: cut-off value: ^{13}C-glucose; t = 60: < 2.0 mmol/l	10
$^{13}C/^{2}H$-glucose test: cut-off value: ratio ^{13}C-glucose / ^{2}H-glucose; t = 45 – 75: < 0.46	12

^{2}H-glucose was simultaneously studied. The differences in ^{13}C-glucose response after ingestion of 25 g ^{13}C-lactose in our current study were similar to the results of our previous study and confirm that lactase non-persistent persons have a residual lactase enzyme activity. This enzyme activity is probably relevant for the physiological response after the consumption of lactose containing dairy products. When the kinetics and 45 min concentration of the reference sugar ^{2}H-glucose in the digester and maldigester group are compared, a significant difference can be observed. This can be explained by taking into account the difference in insulin response between both groups due to differences in the influx of lactose derived glucose (Fig. 4). This is confirmed by the observation

that when a mixture of 26.3 g ^{13}C-glucose and 0.5 g ^{2}H-glucose is consumed by the digester and the maldigester group, the difference in ^{2}H-glucose response between both groups disappears (Fig. 3). Thus it can be concluded that no differences exist in the processing of glucose between both groups. Therefore, the ratio ^{13}C-glucose/^{2}H-glucose concentration in serum both in the digester and the maldigester group exclusively reflects the difference in lactase enzyme activity. The results of this *in vivo* measurement of lactase activity are shown in Fig. 5. A significant difference in the ratio is observed in the first two hours after consumption of the sugars. After that period, the SD of the difference is strongly increasing due to physiological and analytical variations in the ^{13}C-glucose and ^{2}H-glucose concentrations in serum. In this study we used the mean values at 45, 60 and 75 minutes to calculate the ratio. Considering a shorter test period, variations due to gastric emptying will have a distinct influence. In the future we will select a single, optimal sampling time point for practical reasons. The superior results of the ^{13}C/^{2}H-glucose test compared to the previously described ^{13}C-lactose digestion test[8] are illustrated in Fig. 6. In the new test, much less overlap was found between the results of the first and the three other groups. This implies that the discriminative power of the ^{13}C/^{2}H-glucose test is stronger. The improvement that is introduced by the addition of ^{2}H-glucose as a reference is probably mainly due to a correction for the individual variations in the insulin effect. As discussed before, in digesters the glucose influx after lactose digestion causes an insulin response which affects both the ^{13}C-glucose response and the ^{2}H-glucose response. This enhances the difference in the obtained results between both groups. Another advantage of using the ^{13}C/^{2}H-glucose test is the correction for causes of major interference, caused by general transport defects in the intestine due to various reasons Fig.7.

Finally, the outcome of this new test is compared with other clinically used tests to diagnose hypolactasia. In Table 2 the accuracy of the H_2-breath test, the $^{13}CO_2$-lactose breath test[11] and the ^{13}C-lactose digestion test[8] are compared. The genetically determined lactase non-persistence state is taken as the "golden standard". These figures also indicate that the ^{13}C/^{2}H-glucose test is a promising test for future clinical and population studies.

This new test can improve the diagnosis of genetically determined and acquired hypolactasia, although for daily clinical practice, the need of highly sophisticated apparatus will limit general application. Because of its semi-quantitative character, it can also be applied in other areas of research, for instance in studies about the mechanism of lactose intolerance, in developmental aspects of the enzyme lactase and as indicator of intestinal function in pathological states. We also have to realize that the ^{13}C/^{2}H-glucose ratio is the outcome of the physiological digestive function of the total small intestine and therefore has an advantage over the information about the enzyme activity in biopsy material. This is particularly relevant when we are aware of the fact that the change in lactase

activity in the intestinal mucosa in pathological states can have a patchy character[12, 13].

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CHAPTER 7

Lactose intolerance: analysis of underlying factors

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ABSTRACT

Background

We studied the degree of lactose digestion and orocecal transit time (OCTT) as possible causes for the variability of symptoms of lactose intolerance (LI) in a sample of a population with genetically determined low lactase activity.

Methods

Lactose Digestion Index (LDI) was measured by the recently developed ^{13}C-lactose/^{2}H-glucose test. The OCTT was determined using the breath hydrogen test. Based on a 6-h symptom score (SSC) after a challenge dose of 25 g of lactose the subjects were divided into a tolerant group (T: $n = 15$; SSC = 0) and an intolerant group (IT: $n = 28$; SSC 1 - 40). The intolerant group was subdivided according to the severity of symptoms: group ITa ($n = 17$; mild symptoms without diarrhoea) and group ITb ($n = 11$; with diarrhoea).

Results

The LDI was lower in the intolerant group (0.34±0.14) (mean±SD) than in the tolerant group (0.47±0.14) ($P = 0.008$).The OCTT of group IT (60, 30 – 90 min) (median, quartiles) was significantly shorter than that of group T (105, 60 – 120 min) ($P = 0,003$) and was positively correlated with the LDI ($P = 0.012$). In group ITa and ITb the OCTT (60, 30 – 90 min; 60, 26 - 83 min) and LDI (0.30±0.14; 0.39±0.14) were similar.

Conclusions

Lactose digestion capacity, which is determined by the intestinal lactase activity as well as by the OCTT, affects the occurrence of lactose intolerance. However, the major difference in intolerance symptoms is caused by differences in colonic processing of maldigested lactose.

INTRODUCTION

The occurrence and severity of lactose intolerance (LI) after milk consumption is highly variable[1-5]. Variations in the degree of lactose digestion (LD), might be the responsible factor, but a reliable measurement of LD has so far been impossible. In this study, we applied a recently developed method to determine differences in small intestinal LD[6]. We used a dose of 25 g of ^{13}C-lactose, an equivalent of the lactose amount in 500 mL of cow's milk, and performed the study in a population sample of adults with genetically determined low lactase activity (Chengdu, Sichuan province, China). In addition to the degree of digestion, we measured the orocecal transit time (OCTT) and the breath hydrogen excretion (BHE) of this group.

MATERIALS AND METHODS

Subjects

Forty-three (22 female and 21 male, age range 19 - 53 years) Chinese students and lecturers of the Sichuan University, Chengdu, China, were recruited for this study. They were selected from a group of 101 subjects, who had participated in a lactose tolerance test 3 years earlier[7]. The subgroups were one group of tolerant students, one group of intolerant students and one group of lecturers who were all intolerant. From these groups, 15, 14 and 14 subjects, respectively, were randomly chosen. All subjects were apparently healthy and had not been using antibiotics or other drugs affecting intestinal function during the 2 months preceding the study. Verbal informed consent was obtained from all tested subjects. The Ethics Committee of the Sichuan University approved the study.

Experimental protocol

The subjects were asked to refrain from consuming foods naturally enriched in ^{13}C, such as cane sugar, corn products and pineapple, for 3 days before the experiment. After an overnight fast (> 12 h), 25 g of ^{13}C-lactose and 500 mg ^{2}H-glucose dissolved in 250 mL of water was administered orally. The ^{13}C - lactose was obtained from the Dutch Institute for Dairy Research (NIZO), Ede, The Netherlands (Dr. R. van der Meer)[6]. 6,6-^{2}H-glucose, 98 % ^{2}H, was obtained from Isotec Inc. (Miamisburg, Ohio, USA). The subjects remained quietly seated and refrained from smoking during the test period. They were allowed to drink water or tea (without sugar and cream) from 2 h after the substrate consumption. A venous catheter (Becton Dickinson GmbH, Heidelberg, Germany) was placed in an antecubital vein, which allowed repetitive blood sampling. Two mL samples of venous blood were taken 15 min before substrate consumption. Subsequently samples were collected every 15 min for 2 h. The blood-sampling tubes

(Vacutainer, Becton Dickinson GmbH, Heidelberg, Germany) contained sodium fluoride and potassium oxalate. The end-expiratory air was sampled by breathing through a straw into 20-mL vacutainers. Breath-air samples for H_2 determination were collected at 30 and 15 min before the lactose intake. During the first 4 h after the substrate consumption samples were collected every 15 min and during the following 2 h every 30 min.

Symptom score

During the first 6 h after the lactose consumption, the subjects scored the occurrence and severity of symptoms hourly, thereafter at longer intervals (afternoon: 3 h, evening: 5 h, night: 7 h, morning: 5 h, afternoon: 5 h). Flatulence and abdominal cramps were recorded using a ranked scale: 0 = none, 1 = mild, 2 = moderate and 3 = severe symptoms. Also the occurrence of each bowel movement was registered together with its consistency: 0 = normal, 1 = loose, 2 = watery. To weigh the different complaints according to their clinical significance, the scores were multiplied by different factors: 1 for flatulence, 2 for abdominal cramps, 4 for stool consistency. The total symptom score (SSC) is the sum of all scores recorded. The SSCs of 9 and 14 h were slightly higher than the 6-h SSCs in most of the intolerant subjects, but did not result in a different group separation. Therefore the SSC of the first 6 h following lactose ingestion was used for classification of the groups. During this time period no other food was taken and symptoms were most likely related to lactose maldigestion.

Analytical methods

Plasma ^{13}C-glucose and ^{2}H-glucose analysis and calculation of lactose digestion index

The analyses and calculations were performed as described before[6] with one modification: instead of the penta-acetate, butylboronate-acetate derivatization was applied[8]. The $^{13}C/^{2}H$-glucose concentration ratio obtained in this test was the outcome of the physiological digestive function of the entire small intestine. The mean ratio of three time points (45, 60 and 75 min) was defined as LDI. The amount of lactose digested was calculated by multiplying the LDI with the dose lactose administered.

Breath hydrogen determination, calculation of breath hydrogen excretion and orocecal transit time

Breath hydrogen concentration was analyzed as described before[7]. The mean H_2-concentration (parts per million; ppm) of two breath samples before lactose ingestion was taken as the basal value. Subsequent Δ H_2 concentrations were expressed as increment above this basal level. When an increment in excess of

20 ppm above the basal value was sustained, the subject was considered to be H_2-positive. Total BHE was estimated by calculating the area under the concentration-time curve between 0 and 360 minutes. The OCTT was determined by the interval between consumption of the substrate and the time of occurrence of a sustained rise of > 5 ppm above the mean basal value[9,10].

Statistics

All data are expressed as mean ± standard deviation, except for the OCTT-data which are presented as median with quartiles. Differences between groups in the $^{13}C/^{2}H$-glucose concentration ratio, BHE, time to the H_2-peak and age were assessed with the Student *t*-test (unpaired, two tailed). The Mann-Whitney *U* test was applied for comparison of the OCTT data. Correlations were assessed by calculating Spearman's r_s. $P < 0,05$ was considered to be significant. All analyses were performed with the use of SPSS 9.0 for Windows software. (SPSS Inc., Chicago, IL, USA).

RESULTS

Out of the group of 43 subjects, 15 persons did not develop any symptoms (group T) after consumption of the lactose, and 28 persons recorded symptoms of different severity (SSC range: 1 – 40) and were classified as intolerant (group IT). The IT group was subdivided according to the occurrence of loose or watery stools (subsequently referred to as diarrhoea): group ITb ($n = 11$, with diarrhoea) and group ITa ($n = 17$) for those who only recorded flatulence and/or abdominal cramps.

The LDI, representing the degree of lactose digestion, the OCTT (Fig. 1), BHE (Fig. 2) and age of the subjects were compared between the tolerant (T) and the intolerant group (IT) and also between the subgroups with (ITb) and without diarrhoea (ITa) .

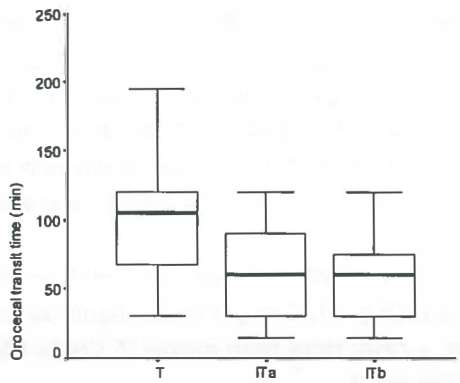

Figure 1. Orocecal transit time after ingestion of 25 g lactose in subjects without (T, $n = 15$), with mild (ITa, $n = 17$) and diarrhoea-predominant (ITb, $n = 10$) symptoms of lactose intolerance after ingesting lactose 25 g.

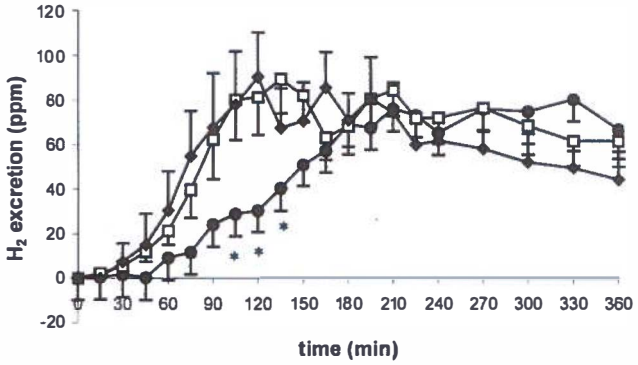

Figure 2. Changes in breath hydrogen excretion after ingestion of 25 g lactose in subjects without (●-●; n = 15), mild (❑-❑; n = 17) and diarrhea-predominant (◆-◆; n = 10) symptoms of lactose intolerance. Values are means± SEM. *P < 0.05

Tolerant vs. intolerant

Table 1. Comparison of the lactose digestion index (LDI), amount of lactose digested, orocecal transit time (OCTT), breath hydrogen excretion (BHE) and age of lactose tolerant (group T) and intolerant subjects (group IT) in response to the ingestion of 25 g lactose

	n	LDI†	amount of lactose digested (g)†	OCTT (min)‡	BHE (AUC 6 h) ppm†	age (years†)
group T	15	0.47±0.15	11.8± 3.9	105 (60 –120)	293± 166	23± 0,5
group IT	28	0.34± 0.14*	8.5± 3.5*	60 (30 – 90)‡*	357± 194§	30± 10*

† Mean ± sd
‡ Median (quartiles)
§ *n* = 27 (one hydrogen negative person)
* Significantly different from group T (*P* < 0,01)

In group T the mean LDI (0.47± 0.15) was significantly higher than in group IT (0.34±0.14) (P = 0.008), indicating the digestion of 11.8± 3.9 g of lactose compared to 8.5± 3.5 g in each group, respectively. All subjects had a positive H_2-breath test result except of one person from group IT. The OCTT of group T (105; 60 –120 min) was significantly longer than that of group IT (60; 30 – 90 min) (*P* = 0.003). The individual values of the LDI and the OCTT of all subjects were positively correlated (r_s = 0.30, *P* = 0.050) (Fig. 3). The mean age of group T (23± 0.5) was lower than that of group IT (30± 10) (*P*= 0.001) and was much less variable.

The BHE did not differ between the groups studied and was not significantly correlated with the LDI (r_s = 0.18; *P* = 0.26) (Fig. 4). Group T had a significantly slower BHE rise (*P* < 0.05) and a later H_2 - peak time than group IT (248± 65, 161± 70 min respectively, *P* = 0.001) (Figure 2).

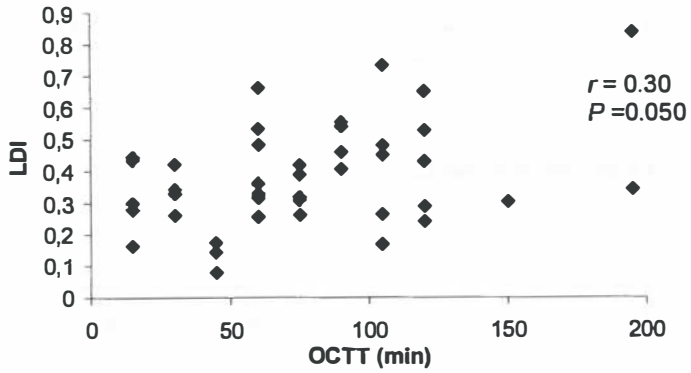

Figure 3 Correlation between orocecal transit time (OCTT) and lactose digestion index (LDI) in 42 subjects with genetically determined low lactase activity

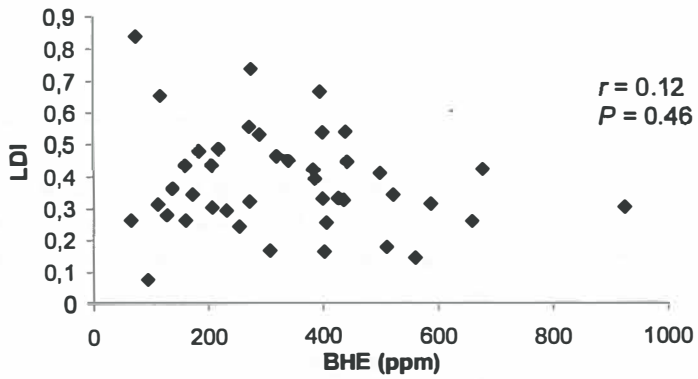

Figure 4 Correlation between breath hydrogen excretion (BHE) and lactose digestion index (LDI) in 42 subjects with genetically determined low lactase activity

Diarrhoea versus no diarrhoea

Table 2. Comparison of the lactose digestion index,, amount of lactose digested, orocecal transit time, breath hydrogen excretion and age of subjects with mild (no diarrhoea) and diarrhoea-predominant (diarrhoea) lactose intolerance symptoms in response to the ingestion of 25 g lactose

	n	LDI*	amount of lactose Ingested (g)*	OCTT (mIn)†	BHE (AUC 6 h) ppm*	age (years*)
no diarrhea	17	0.30± 0.14	7.5± 3.5	60 (30 – 90)	358± 225	28± 10
diarrhea	11	0.39± 0.15	9.8± 3.8	60 (26 - 83) ‡	357± 140‡	34± 10

* Mean ± SD
† Median (quartiles)
‡ *n*=10 (one hydrogen-negative person)
LDI, Lactose Digestion Index; OCTT, orocecal transit time; BHE, Breath Hydrogen Excretion

The intolerant subgroups with and without diarrhoea did not differ statistically in any parameter studied. In both groups also the BHE rise and the time to the H_2 -peak were identical (155± 60 and 171± 87 min. respectively, $P = 0.57$) (Fig. 2).

Reproducibility of (in)tolerance

Table 3. Reproducibility of lactose intolerance symptoms

	lactose challenge I	lactose challenge II
Tolerant	16/16	10/16 SSC = 0
volunteers		6/16 SSC < 5, no diarrhoea
group ITa	NA	
group ITb	9/9	7/9 diarrhoea
		2/9 no diarrhoea (SSC < 3)

To test the reproducibility of symptoms 16 lactose tolerant subjects (previous study)[7] and nine subjects from group ITb underwent a second lactose (in)tolerance test after an interval of more than 6 months, using the same dose of lactose. In this repeated test 10/16 (62 %) tolerant subjects had no complaints, while 6/16 (38 %) developed minor symptoms (SSC < 6) without diarrhoea. In group ITb, 7/9 (78 %) again suffered from diarrhoea and 2/9 (22%) had only minor symptoms (SSC < 3) in the second test.

DISCUSSION

All subjects belonged to a population with genetically determined low lactase activity. The classification of (sub)groups was based on differences in symptoms during the 6-h post- ingestion period. In 35% of the tested persons (group T, SSC = 0) no complaints were recorded. From the intolerant group (group IT, SSC 1 - 40) 61% recorded mild complaints (group ITa) and 39 % severe symptoms (diarrhoea) (group ITb). These data indicate that in a population with genetically determined low lactase activity, a considerable number of persons is able to consume approximately 0.5 L. of milk without any symptoms of lactose intolerance, which is in agreement with the results of previous studies[11-13]. A repeated lactose tolerance test in two subgroups (16 tolerant subjects and nine persons from group ITb) showed that the status of (in)tolerance remained unchanged in most of the tested individuals (Table 3). This suggests that host related factors play a major role in lactose (in)tolerance and might be traceable.

In group T we found a mean LDI of 0.47± 0.15. Despite this relatively high LDI all members of this group had a positive hydrogen breath test result, which confirms lactose maldigestion. The LDI of group IT was significantly lower (0.34± 0.14) than that of the tolerant group. These results demonstrate that lactose tolerance in a group of persons with genetically determined lactase non-persistence is caused by a higher degree of LD.

The degree of digestion of a dose of lactose is determined by the (residual) activity of the small intestinal lactase activity and by the time available for lactose hydrolysis in the small intestine, which is reflected by the OCTT. Several studies could demonstrate a positive influence of a prolonged OCTT on the severity of lactose intolerance[14-17]. The mechanisms involved in this phenomenon could until now only be evaluated by indirect measurements, such as the hydrogen breath test[14,16,18]. Using this technique the effects of small intestinal digestion and colonic fermentation of the substrate could not be analyzed separately. We therefore examined the relationship between transit time and LDI; a direct reflection of LD. The weak positive correlation (Fig.3) between the OCCT and the LDI indicates that OCTT contributes to the degree of LD. Also in the present study we showed that a fast OCTT clearly contributes to lactose intolerance since the OCTT of group IT (60, 30 – 90 min) was significantly faster than that of group T (105, 60 - 120 min) (Table 1). The demonstrated effect of the OCTT on lactose digestion and its important role in lactose (in)tolerance raises the question as to what are the underlying mechanism of the differences in transit time in the subjects of our study. Experiments with different non-absorbable sugars showed that malabsorption can accelerate small intestinal transit time[13,19-21]. This is ascribed to an increased intestinal liquid volume because of the osmotic effect of the malabsorbed sugar, which results in intestinal distension and stimulation of motility[20]. However, it is questionable whether this effect can cause differences in transit time within a population with low lactase activity. The measured differences in amounts of undigested lactose (± 3,3 g) were small in this study and the resulting differences in osmotic effect are not likely to have resulted in the observed large differences in transit time. A possible explanation for the different rate of transit could be a host-specific factor, independent of lactase activity level, as large inter-individual differences in small intestinal transit time seem to exist[22-24], or, alternatively, an activation of the intestinal motility by carbohydrates like fructose or lactose by unknown mechanisms in susceptible persons. These phenomena might be related to the underlying factors in the irritable bowel syndrome (IBS)[25,26] or in recurrent abdominal pain syndrome (RAP) in children. This implies that symptoms of LI and IBS or RAP could have partly similar underlying causes and might be difficult to differentiate in clinical diagnosis. Prolonging intestinal transit, by changing the matrix in which lactose is consumed or by pharmacological intervention in the clinical situation, could therefore prevent possible symptoms in persons

with mild LI.

Breath hydrogen excretion is often used as indicator of the degree of lactose digestion[14,27], but the quantitative aspect of this parameter is disputed[28]. The low LDI in group IT implies a higher amount of lactose reaching the colon for fermentation, however no difference in BHE between group T and IT (Table 1) and no correlation of the LDI with the BHE were observed (Fig. 4). Thus, our data confirm that BHE does not reflect the amount of undigested lactose. However, it appears that group T had a significantly slower rise (Fig.2) and a later H_2-peak than group IT (248± 65, 161± 70 min, respectively, $P = 0{,}001$). This implies that a slow rate of colonic delivery coupled with a smaller amount lactose leads to an extended slow rise in H_2 production which could be the cause of the asymptomatic state.

The LDI as well as the OCTT were not different between groups ITa and ITb (Table 2). From this observation it can be concluded that the reported diarrhea is not caused by a faster delivery or/and a larger quantity of lactose entering the colon, but must be due to inadequate colonic processing of lactose. A similar finding was reported by Clausen *et al*[29] who observed that some but not all of their subjects responded with diarrhea to a given amount (40 en 80 g) of the unabsorbable sugar lactulose. The mechanism of adaptation to continued lactose ingestion[30,31] could also imply involvement of the colon in the reduction of lactose intolerance symptoms. Adaptation, however, did not seem to play an important role in our study, as a considerable proportion of group T (47 %) and ITa (47 %) did not consume milk regularly (data not shown). Therefore, the colonic characteristics contributing to resistance to diarrhoea seem to be host-specific. The nature of these colonic factors are currently subject of intensive microbiological research.

Colonic (resistance) factors could in theory be manipulated by the use of pre- and probiotics. Our observations could thus lead to changed strategies in the prevention of lactose intolerance, and thereby might have an impact on the nutritional calcium supply in most parts of the world.

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Chapter 8

Lactase activity in small bowel biopsy specimen is not an accurate reflection of overall intestinal lactose digestion capacity

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Submitted

SUMMARY

Background

Lactase activity (LA) in a small intestinal bowel biopsy (SBB) specimen is frequently used as the indicator for the intestinal capacity to digest lactose, but its relation with the digestive capacity has not been established. We compared lactase activity in SBB with the results of a quantitative lactose digestion test.

Method

We compared LA in 18 children aged 0.8-10.9 yr (mean 3.9, SD 2.4) suspected of small bowel mucosal damage to the lactose digestion index (LDI), the lactose H_2 breath test and the histology in the biopsy.

Findings

In 5/6 patients with normal histology a normal LA was shown, in 1/6 the LA was low. In all 6 the Lactose Digestion Index (LDI) was normal (> 0.45) and the H_2 test was negative. In 3 of 5 patients with minor histological changes LA and LDI were both normal, with a negative H_2 test. One patient with medium histological changes had a low LA with a normal LDI and a negative H_2 result. In 6 patients with severe mucosal damage LA was low in 5/6, but 3/5 demonstrated a normal LDI while the H_2 test was negative in 3/6. One patient with severe mucosal damage showed a normal LA with a negative H_2 breath test and a low LDI.

Interpretation

Our results indicate that the total lactose digestive capacity can remain adequate despite low Lactase activity in a SBB. Extrapolation of the results of measurement of low LA in SBB specimens to the overall digestive gut function may not be reliable.

INTRODUCTION

Lactose (Galactose-ß (1-4)-glucose) is digested in the small bowel into glucose and galactose by the brush border enzyme lactase. A decrease in the activity of this enzyme is considered an indicator of low lactose digestive capacity. Measurement of lactase activity (LA) in a small bowel biopsy (SBB) specimen is considered the "gold standard" for determination of lactose digestive capacity. All other diagnostic tests are validated against this standard[1-13], their sensitivity and selectivity have been disappointing. Recent data from Maiuri et al[14] indicate that lactase activity in SSB's from patients with mucosal damage actually has a patchy distribution, low and normal lactase activity can be demonstrated in different biopsies from the same patient. It can therefore be questioned if the lactase activity in small bowel biopsy material is the true reflection of overall lactose digestion capacity. We recently developed a lactose digestion test, using ^{13}C-labelled lactose as a test substrate to measure lactase activity in vivo. We added ^{2}H- glucose as a reference substrate to correct for individual variations in gastric emptying time, absorption and post-absorptive metabolism[1]. We validated this test by comparing healthy Chinese subjects who genetically exhibit low lactase activity with subjects of Dutch origin who are known to persist in high lactase activity[1]. The ratio between the ^{13}C-glucose and ^{2}H-glucose concentration in plasma was shown to be different between the Chinese and the Dutch subjects, indicating the difference in intestinal lactase activity. This ratio reflects the degree of digestion of the consumed dose of ^{13}C-lactose and is referred to as the Lactose Digestive Index (LDI).

Here we compare the in vivo test for measuring lactose digestive capacity (LDI) with lactase enzyme activity measured in small intestinal biopsies, taken for different clinical reasons, as well as the Lactose H_2 breath test, currently the most frequently used non-invasive lactose digestion test.

MATERIAL AND METHODS

Patients

18 consecutive patients (7 girls and 11 boys) who were scheduled in our hospital in 2001 for a small bowel biopsy for clinical reasons were included in this study. Their clinical details, including nutritional status and indications for the biopsy are shown in Table 1. All individuals originated from a Caucasian population with a high prevalence of genetically determined lactase persistence.

Test protocol

The ^{13}C-lactose/^{2}H-glucose test was performed directly before the SBB procedure. Patients refrained from consumption of ^{13}C-enriched foods like cane sugar, pineapple and corn derived products during the three days preceding the test. The subjects fasted for at least 8h prior to the test whereby only consumption of water was allowed.

Substrate

We used naturally labelled ^{13}C-lactose (^{13}C-abundance 1.096%, δ ^{13}CPDB – 13,7‰) derived from milk of cows fed cattle fodder corn for 5 weeks, produced by the Netherlands Institute for Dairy Research, Ede, The Netherlands. (Kindly provided by dr. R. van der Meer)[2] as the test substrate. ^{13}C-lactose (2g/kg body weight) was consumed in a 20% aqueous solution. As a reference substrate 6,6-^{2}H-glucose (98% ^{2}H) was used (6,6-^{2}H-glucose was added to the substrate in a dose of 0.04 g/kg body weight). This was purchased from Isotec Inc, Miamisburg, OH, U.S.A.

An intravenous catheter (Becton Dickinson GMBH, Heidelberg, Germany) was put in place to facilitate repetitive blood sampling and to administer anaesthetics during the biopsy procedure. Blood samples (1 mL) were taken before substrate consumption and 15, 30, 45, 60, 75, 90, 105 and 120 minutes afterwards, and were injected into blood-sampling tubes (Vacutainer ®, Becton Dickinson GMBH, Heidelberg, Germany), which contained sodium fluoride and potassium oxalate. Post sampling handling was identical to the procedure described before[1].
Breath samples were collected in 20 ml syringes before substrate consumption and half hourly 2 hours afterwards to measure H_2 concentration as described previously[2]. None of the study subjects had clinical symptoms of lactose intolerance like diarrhoea or vomiting during the test.

Biopsy

The biopsy was performed under general anaesthesia using an Olympus GIF 160 endoscope. Biopsies were taken from the distal duodenal mucosa.

Ethical considerations

The Medical Ethics Committee of the Groningen University Hospital approved this protocol. Informed consent was obtained from the patients and the parents in accordance with the principles expressed in the Declaration of Helsinki.

MEASUREMENTS

Lactase activity

The lactase activity was measured with a modified Dahlqvist method and expressed in units per gram protein[15]. Normal values are above 10U/g protein, as described before[2].

Breath hydrogen concentration

3 ml of the 20 mL collected air was injected to a 3 mL Vacutainer tube ® (Terumo Europe NV, Leuven, Belgium). The tubes were stored at room temperature and analysed within the first week after the test. Under these storage conditions the quality of breath samples has been proven to remain unimpaired[16,17]. The breath samples were analysed on a HP 5880 gas chromatograph for H_2 concentration[18]. A positive test result was defined as an increase in concentration above basal H_2 values (delta H_2) of more than 20 ppm at any time point during the test period[2].

^{13}C-glucose and ^{2}H-glucose concentrations in serum

The analyses were performed as described previously[1]. Briefly, serum was deproteinised and glucose was derivatised to the penta-acetate derivative.^{13}C-glucose enrichment was measured with GC/combustion/IRMS (Delta S/GC, ThermoFinnigan, Bremen, Germany) and ^{2}H-glucose enrichment was measured with GC/MS, using a SSQ7000 quadrupole instrument (ThermoFinnigan, San Jose, CA, U.S.A.)

The mean ratio between the serum ^{13}C-glucose and ^{2}H- glucose concentrations at 45,60 and 75 minutes after substrate consumption were used to calculate the Lactose Digestion Index, which is the ratio of the concentrations of both glucose markers in serum expressed as a percentage of the consumed dose of tracer. Multiplication of the LDI with the consumed dose of lactose results in the digested amount of lactose as shown in table 1.

Histology

The histopathological examination was done by one pathologist who had no prior knowledge of clinical or laboratory data. The biopsies were graded 0 (normal) to IIIC (total villous atrophy) according to the modified Marsh classification[19].

The Lactose Digestion Index

In a previous study we showed that a LDI of >0.45, indicating that more than 45% of ingested lactose is digested in the small intestine, discriminated between a lactase deficient and a lactase persistent population[1]. A LDI cut off of 0.45 therefore was used in this study.

RESULTS

In 6 of the 18 individual biopsy specimens a normal mucosal architecture was shown (grade 0), 5 of them had a normal lactase activity (mean 33.5 U/g protein, SD 13.6) in their specimens. In one of the patients the measured LA was low (2.8 U/g protein), despite normal histology. However, in all 6 subjects the LDI was higher than 0.45 (mean 0.69, SD 0.07). The results of the H_2 breath test were also normal in these individuals (Table 1).

In 12 biopsies mucosal damage was demonstrated, ranging from the modified Marsh grades II to IIIC. In 3/5 patients with minor histological changes (grade II) both the lactase activity and the LDI were normal together with a normal H_2 breath test result. In 2 cases of grade II damage the LA was low (3.6 and 5.6 U/g), both had an abnormal H_2 breath test result, while the LDI was low in one of them (0.31) and normal in the other one (0.50).

In the patients with grade IIIA-IIIC mucosal damage (n =7) only one patient demonstrated a normal LA (13.4U/g protein). This patient had a normal H_2 test and a low LDI (0.30).

In the other 6 the LA was low (mean 4.7 U/g protein, SD 3.9). 2 of them had a normal H_2 breath test result. In 3 of them the low LA was associated with a normal LDI and in the other 3 the LDI was low.

Pat	Indication	Weight/l # (SD)	Length/a¶ (SD)	Histology (grade)	L A**	H_2BT††	LDI‡‡	Digested actose dose (g/kg bw)
1	CD*, no diet	-1	-2	IIIC	0	neg	0.62	1.2
2	CD*, gluten provocation	0	0	IIIC	0.7	pos	0.23	0.48
3	CD*, no diet	-1	0	0	2.8	neg	0.68	1.4
4	CD*, gluten provocation	0	0	IIIB	3	neg	0.75	1.5
5	CD*, diet	0	+1	II	3.6	pos	0.31	0.6
6	CD*, no diet	-2	0	IIIC	4.9	pos	0.1	0.21
7	CD*, diet	0	-1	II	5.6	pos	0.5	1
8	CD*, no diet	-2	0	IIIA	9.4	neg	0.59	1.26
9	CD*, no diet	-1	-2	IIIB	9.9	pos	0.57	1.1
10	CND†	-2	-2	IIIB	13.4	neg	0.3	0.59
11	Suspected Lactose intolerance	+1	-1	II	15.3	neg	0.71	1.42
12	CD*, diet	-1	0	II	15.5	neg	0.62	1.24
13	Suspected Lactose intolerance	+2	0	0	19.3	neg	0.64	1.27
14	RAP‡	0	-2	0	19.9	neg	0.56	1.09
15	RAP‡	-1	0	II	21.1	neg	0.73	1.46
16	FTT§	-2	-2	0	29.8	neg	0.74	1.49
17	CD* ,gluten provocation	-1	0	0	45.3	neg	0.75	1.52
18	CND†	-2	-1	0	53.0	neg	0.74	1.57

* Celiac disease
† Chronic Non-specific Diarrhea
‡ Recurrent Abdominal Pain
§ Failure to thrive
#Weight related to body length (Standard Deviation)
¶ Body Length related to age (Standard Deviation)
** Lactase Activity (Units/g protein)
†† Hydrogen Breath Test
‡‡ Lactose Digestion Index

Table 1:
Characteristics and test results of 18 pediatric patients undergoing Small Bowel Biopsy

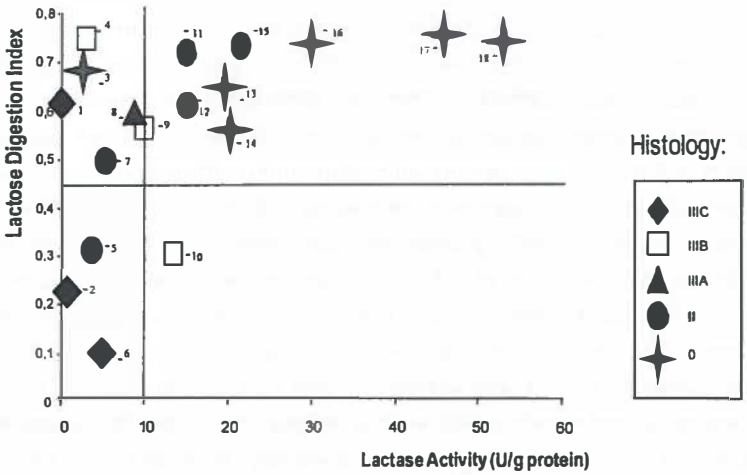

Figure 1. Lactose Digestion Index versus Lactase Activity in relation to grade of histological changes in SBB specimens (numbers refer to patient numbers in table 1)

The results of the LDI, the histological mucosa grading and the lactase activity are shown in figure 1.

DISCUSSION

Impaired lactase activity leading to incomplete small intestinal lactose digestion can cause intolerance symptoms like bloating, flatulence, abdominal pain and diarrhoea. At present, impaired lactase activity is diagnosed by decreased lactase activity in a small bowel biopsy. This measurement determines the lactose hydrolysing capacity in a biopsy in vitro, but the relation between these results and the in vivo lactose digestive capacity of the whole small intestine is unclear, because lactase activity in a damaged intestinal epithelium could have a patchy distribution[14]. In the past decades many techniques have been developed to diagnose diminished lactase activity: (Lactose Tolerance Test, Lactose Tolerance Test with ethanol addition, H_2 breath test, $^{14}CO_2$ and $^{13}CO_2$ breath test[2,3,4,5,10]). However, none of these methods has been shown to reliably quantify total lactose digestive capacity. When intestinal lactose digestive capacity is underestimated, dietary lactose restriction as a consequence will be too rigid. Since lactose is an important component of dairy products, intake of other dairy food components as proteins, minerals and vitamins will be reduced also. As an alternative for dietary lactose restriction the consumption of lactase preparations in addition to consumption of lactose containing foods can be prescribed. This significantly increases health costs.

Our recently developed ^{13}C-lactose/^{2}H-glucose test is based on the relation between the absorption of lactose derived monosacharides (glucose and

galactose) and the absorption of a reference monosaccharide (^{2}H-glucose). This method enables to determine the percentage of the consumed lactose that is first hydrolysed and then absorbed. By adding the glucose tracer, a correction is made for glucose malabsorption. The only difference between the ^{13}C and ^{2}H-plasma glucose concentration is the lactase activity. When we evaluated our technique in individuals with genetically determined differences in lactase activity (lactase persisting and lactase non-persisting subjects), the cut off level for full discrimination between both groups was documented to be 45% of dose (LDI 0.45) using a lactose dose of 25 gr. In groups with genetically determined differences in lactase activity we can expect a homogenous distribution of lactase activity over the small intestine and not a patchy distribution and thus a correlation between the lactase activity in SBB material and the LDI. For ethical reasons we could not confirm this with a biopsy in this healthy population.
Small intestinal mucosal damage, due to a variety of causes like celiac disease, radiation damage, gastroenteritis and cytostatic treatment, can also result in decreased lactase activity. Decreased lactase activity in SBB material does not necessarily represent the physiological digestive capacity of the small intestine, because of the patchy character of the lesions. Our results indicate that the physiological lactose digestive capacity can remain high despite measured low LA in a biopsy specimen. The H_2 breath test results (H_2BT) in the patients with a low lactase activity in the biopsy (n=9) were non conclusive: In 4/9 there was a normal and in 5/9 an abnormal H_2BT. All 4 patients wit a normal H_2BT had a normal LDI (>0.45). However, 2 of the 5 patients with an abnormal H_2BT also had a normal LDI (0.50 and 0.57). In all patients with a high SBB lactase activity the H_2 breath test results were normal.
In only one of the patients with a lactase activity of more than 10U/g protein (patient number 10, Table 1) the lactose digestive capacity index was impaired (0.30). The H_2 breath test result in this boy was normal. This patient has an un-specified cellular energy deficit disease as shown by mitochondrial respiratory chain measurements in a muscle biopsy. The intracellular processing of glucose is therefore suspected to be impaired and for that reason the results of the LDI in this patient have to be interpreted with caution.
We believe that the discrepancy in the results of the LDI and the LA is due to the patchy distribution of the small intestinal mucosal damage. This means that the site where the SBB is taken is crucial for the diagnosis of a low lactase activity. Only when there is extensive mucosal damage, a low biopsy lactase activity may be considered representative for reduced overall intestinal lactose digestive capacity.
We recommend using a lactose digestive capacity test and not an intestinal biopsy to detect a reduced lactose digesting capacity. Negative effects of restrictive dietary regimens (costs, nutritional status) may be avoided.

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Chapter 9
Summary and General Discussion:

Milk and dairy products are important nutrients in the Western diet. When milk products are eliminated consumption of alternative calcium, protein and vitamin containing products is necessary. To avoid unnecessary dietary measures when lactose intolerance is suspected, reliable measurement of the lactose digestive capacity is essential. Although many diagnostic methods have been developed in the past decades, none of them has shown to be capable to accurately determine the amount of lactose digested. An overview of techniques currently in use including their differences, accuracy and diagnostic reliability is presented in chapter one.
In the studies described in this thesis we aimed to develop a more precise method to measure lactose digestion in humans. In addition to a higher diagnostic accuracy, our new test should also be easy applicable in clinical practice and allow for studies of the mechanisms involved in lactose digestion.

^{13}C -lactose $^{13}CO_2$ breath test (Chapter 2):
This test is based on the principle that consumed labelled ^{13}C-lactose will be hydrolysed into ^{13}C-glucose and ^{13}C-galactose by lactase. Both labelled monosaccharides are transported to the liver after intestinal absorption. In the liver ^{13}C-galactose is converted to ^{13}C-glucose. Both ^{13}C-glucose molecules, the absorbed ^{13}C-glucose and the ^{13}C-galactose converted into glucose, will be metabolised in body cells leading to the production of $^{13}CO_2$ which is finally exhaled. The amount of exhaled $^{13}CO_2$ therefore represents the amount of ^{13}C-lactose that is successfully hydrolysed, absorbed and metabolised. In our study as described in chapter 2 we demonstrate that the results of the $^{13}CO_2$ test in children better correlate with the measured lactase activity in small bowel biopsy specimens than the results of the lactose H_2 breath test. This diagnostic improvement however was not large enough to justify introduction of the test in clinical practice. This may have been explained by the fact that under standard test conditions (which is quietly seated) only a small part of the substrate is oxidised, resulting in a $^{13}CO_2$ recovery of less than 25% of dose. Besides, under circumstances of lactose malabsorption, undigested ^{13}C-lactose will be fermented in the colon. This fermentation process leads to production of $^{13}CO_2$, which is also exhaled. This phenomenon leads to an overestimation of ^{13}C-lactose hydrolysis. To quantify the effects of these mechanisms on the outcome of the test we studied the effect of physical exercise as well as colonic carbohydrate fermentation.

Effect of increase of physical activity on the results of the $^{13}CO_2$ breath test (Chapter 3):
We performed the $^{13}CO_2$- breath test with ^{13}C-lactose substrate in healthy young adults under standard resting test conditions. One of the subjects had a non-symptomatic low lactase activity indicated by an abnormal H_2 breath test without

clinical symptoms. The $^{13}CO_2$-test result of this individual was indistinguishable from those of the other individuals with normal lactose digestion (indicated by a normal H_2 breath test). We repeated the test in the same individuals under exercise conditions (bicycling, 50 Watt for 4 hours). The $^{13}CO_2$ breath test of the subject with low lactase activity was clearly indicative for insufficient lactose hydrolysis during exercise, which demonstrates that, in resting healthy individuals, the level of glucose oxidation is the rate-limiting step for conversion of absorbed carbohydrates to CO_2. Increase in the oxidative metabolism can enlarge the percentage of absorbed glucose that is oxidised. Using this principle can make the $^{13}CO_2$ breath test more sensitive. Due to practical limitations however it is not easy applicable in (paediatric) clinical practice.

Effect of colonic fermentation of carbohydrates on the $^{13}CO_2$ breath test result (Chapter 4):

Since fermentation of carbohydrates in the colon not only leads to H_2 but also to CO_2 production, this can influence the sensitivity of CO_2 breath test. To determine the quantity of disturbance of this colonic factor, we studied the CO_2 production in different individuals using different amounts of carbohydrate as substrate. The availability of the non-digestible ^{13}C-lactulose makes it possible to quantify the $^{13}CO_2$ and H_2 production, since all consumed lactulose will reach the colon. Therefore it is possible to relate the amount of carbohydrate in the colon to the amount of gasses resulting from fermentation of the substrate. The fate of undigested lactose in the colon is essentially the same as that of lactulose. To extrapolate the $^{13}CO_2$ excretion rate in breath derived from colonic fermentation from a known amount of carbohydrate substrate to the total recovery of $^{13}CO_2$ in ^{13}C-lactose digestion tests, we created a calculation model. We simulated the effect of increasing lactose maldigestion rates on the $^{13}CO_2$ production in the colon by use of incremental doses of ^{13}C-lactulose. The results showed that both the H_2 and $^{13}CO_2$ production varied strongly interindividually and intraindividually, but the amounts of produced gasses are on the average large enough to influence the result of the ^{13}C-lactose breath tests. No dose-response relationship between the amount of ^{13}C-lactulose and the produced quantity of H_2 and $^{13}CO_2$ was observed. We found that low quantities of undigested ^{13}C-labelled carbohydrate can also lead to production of relatively large amounts of $^{13}CO_2$ in the colon. This $^{13}CO_2$ production can cause an overestimation of lactose digestive capacity using $^{13}CO_2$ breath tests, because the $^{13}CO_2$ originating from fermentation of non-digested lactose is added to the $^{13}CO_2$ from digested and metabolised substrate. A positive correlation between H_2 and $^{13}CO_2$ excretion was found, which means that in instances of high H_2 production from a given dose of lactulose $^{13}CO_2$ production was also high. Under these conditions the H_2 test is more reliable than the $^{13}CO_2$ test (which in that case can give unreliable results due to high colonic $^{13}CO_2$ production as explained before). The $^{13}CO_2$

breath test however is more reliable under circumstances of low colonic gas production, because the disturbing effect of colonic $^{13}CO_2$ on the test will then be low. The H_2 breath test will be less reliable under such conditions, because undigested lactose reaching the colon will not result in production of large quantities of H_2. However, the lack of reproducibility of this "colonic gas production" state in repeated test and between individuals and the lack of a consequent dose response relation makes the ^{13}C-lactose breath test not suitable for further use in clinical applications.

^{13}C -lactose-^{13}C -glucose test (Chapter 5):

The use of ^{13}C -labelled substrate makes it possible to discriminate between glucose derived from hydrolysed and absorbed lactose (^{13}C-glucose) and glucose originating from the body stores (i.e. unlabelled glucose) in blood. Analysis of the rise in concentration of ^{13}C -glucose after consumption of ^{13}C-lactose is therefore indicative of lactase activity and subsequent absorption of glucose and galactose. This test method was validated in two adult population samples, one from Dutch and the other from Chinese origin with a genetically determined persistent or low lactase activity. The difference in lactase activity between these two groups was adequately demonstrated by the results of the test. However, important variations in the time point of the maximal ^{13}C —glucose concentration were found. This might have been caused by individual variations in gastric emptying rate as well as small intestinal transit time. Therefore we expect that the diagnostic quality of this test may be further improved when the disturbance of variations in gastric and intestinal transit time is corrected for.

^{13}C -lactose ^{13}C/^{2}H-glucose test (Chapter 6):

To compensate for transit time variations, we added ^{2}H-glucose to the test substrate. This monosaccharide is subject to the identical gastrointestinal process as the lactose substrate, with the exception of lactose hydrolysis by lactase. Therefore it can act as internal reference. Analysis of the ratio of ^{13}C- and ^{2}H -glucose concentration in blood at different time points after substrate consumption is thus indicative for functional intestinal lactase activity.

This method was also used in two population samples with genetically determined differences in lactase activity, i.e. healthy young Chinese (with lactase non-persistence) and Dutch with lactase persistence. We found a complete separation of both groups with a cut off level of 0.45 for the ratio of ^{13}C- and ^{2}H- glucose concentration between 45 and 70 minutes after substrate consumption. This ratio is further referred to as the Lactose Digestion Index (LDI).

This relatively simple test method is suited for clinical purposes, even in children. Using this new technique we studied the relation between the occurrence of gastrointestinal symptoms after lactose consumption in relation to the amount of digested lactose.

Lactose intolerance: analysis of underlying factors (Chapter 7):
In Chinese adults with low lactase activity the gastrointestinal symptoms occurring after consumption of lactose were recorded. Symptoms were then compared to the results of the ^{13}C-lactose/^{2}H-glucose test. In 35% of subjects no symptoms occurred, while 40% experienced mild gastrointestinal complaints. Only 25% of individuals suffered from diarrhoea. Our data indicate that even in a population with genetically determined low lactase activity, a considerable number of persons are able to consume approximately 0.5L of milk without any symptoms of lactose intolerance. Differences in the residual lactase activity between individuals from the symptomatic and the asymptomatic group were demonstrated by the results of the Lactose Digestion Index. The lactose digestion in the lactose tolerant persons without symptoms was significantly higher (mean LDI 0.47, SD 0.14) than in the symptomatic individuals (0.34 +/- 0.14) ($P< 0.01$). Furthermore the oro-coecal transit time was longer in the tolerant (mean 105 minutes, 60-120 min) than in the intolerant individuals (60, 30-90 min). Both lactase activity and oro-ceacal transit time therefore contribute to the occurrence of symptoms after lactose consumption. However, the observed differences in severity of symptoms within the intolerant group suggest that other factors like for example the colonic processing of maldigested lactose may also be of influence, since no significant differences in lactose digestion index or oro-coecal transit time were observed between individuals with mild or with severe symptoms (diarrhoea).

Lactose digestion and mucosal damage (Chapter 8):
Small intestinal mucosal damage can cause a decrease in lactase activity. We used the ^{13}C-lactose/^{2}H-glucose test to determine the functional rest capacity of lactase in children with mucosal damage. The results of the test were related to the measured lactase activity in small bowel biopsy specimens. In all but one of the children with a normal lactase activity, the LDI was normal. In the patient with normal lactase activity but an abnormal LDI an intracellular glucose-handling problem (mitochondrial respiratory chain defect) is the most probable explanation for this discrepancy. In the children with a low lactase activity in the biopsy, the LDI remained normal in 5 out of 9, indicating that the overall lactose digestion of the intestine is less impaired than presumed on the basis of the biopsy results. Therefore the measured lactase activity in a biopsy cannot be extrapolated to the real functional lactase activity. When dietary advises with respect to lactose restriction in patients with mucosa damage are contemplated, determination of lactose digestive capacity with the ^{13}C-lactose/^{2}H-glucose test provides more reliable information than the biopsy. The use of this test can thus prevent unnecessary dietary lactose restriction in such patients.

We conclude that the ^{13}C-lactose $^{13}CO_2$ breath test is not reliable enough to be used for clinical purposes, because of the influence of variations in glucose oxidation and colonic gas production. Measurement of a rise of ^{13}C-glucose concentration in blood after ^{13}C-lactose consumption better reflects intestinal lactase activity, but is subject to variations in gastrointestinal transit time and glucose turnover. Correction for these variations by adding ^{2}H-glucose to the ^{13}C-lactose substrate is the most reliable method to determine intestinal lactase activity *(in vivo)*.

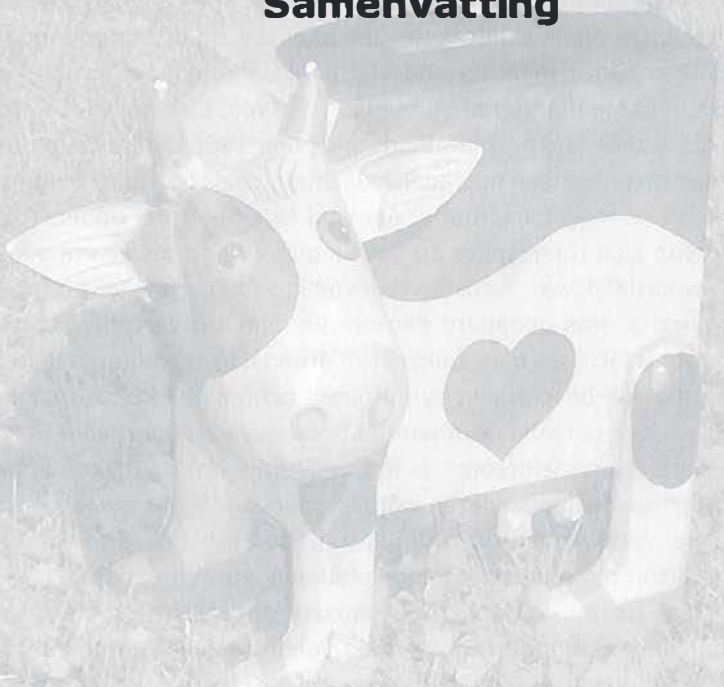
The occurrence of gastrointestinal symptoms after consumption of lactose differs, even in individuals with genetically determined low lactase activity. Asymptomatic individuals had a higher lactose digestive capacity and a longer gastrointestinal transit time when compared to symptomatic subjects, but in symptomatic subjects the severity of symptoms was not related to differences in lactose digestive capacity or transit time. Further research has to reveal the cause of this phenomenon.

The effect of mucosal damage on impairment of intestinal lactose digestion in most of our studied cases was less severe than predicted from lactase measurement in small bowel biopsy. This can be explained by patchyness of mucosal lesions and has to be taken into consideration when dietary advises with respect to lactose intake are given in such patients.

The ^{13}C-lactose/^{2}H-glucose test is a reliable and practical test to study lactose digestion in humans. Therefore it has the potential to further study mechanisms that are involved in biology and pathology of lactase activity, like posttranscriptional factors in lactase enzyme expression, intestinal transit time and colonic adaptation to small intestinal lactose maldigestion. Adequate measurement of the digestive capacity leads to tailored dietary advises and thus can avoid the prescription of unnecessary lactose free or lactose-restricted diets. Not only treatment results but also strategies to improve digestive capacity and diminish adverse symptoms related to lactose consumption may be evaluated.

Finally the test can be used to study lactose digestive capacity in different populations. It may thus be possible to elucidate the relation between genotype and phenotype of lactase expression.

Samenvatting



Samenvatting:
In de eerste maanden na de geboorte gebruikt het kind slechts één soort voeding: (moeder)melk. Na de zoogperiode wordt melk geleidelijk vervangen door andere voedingsmiddelen. De mens is het enige zoogdier dat na de zoogperiode melk kan blijven drinken. Deze melk kan bij voorbeeld afkomstig zijn van koeien, schapen, geiten of kamelen. Vooral bij mensen uit West Europa is melk drinken een gewoonte die al zeer lang bestaat. Sinds ongeveer 1960 is het echter duidelijk geworden dat sommige mensen maagdarmklachten, zoals buikpijn, winderigheid en diarree, krijgen na melkconsumptie. Aanvankelijk heeft het onderzoek naar de oorzaak hiervan zich toegespitst op het vinden van de melkcomponent die hiervoor verantwoordelijk was. Naast overgevoeligheid voor melkeiwit (koemelk eiwit allergie) werd al snel geopperd dat een verminderd verteringsvermogen voor het koolhydraat lactose (melksuiker) ten grondslag zou kunnen liggen aan de klachten. Omdat de beschreven symptomen echter niet specifiek zijn, dat wil zeggen ook andere oorzalen kunnen hebben, zoals bij voorbeeld buikgriep of een "prikkelbare darm syndroom", is het belangrijk om de juiste oorzaak te bepalen voordat behandelingsadviezen worden gegeven. Immers, wanneer melk uit de dagelijkse voeding wordt weggelaten, moeten energie, mineralen, vitamines en eiwitten uit andere voedingsmiddelen worden gehaald.
Melksuiker bestaat uit de componenten glucose en galactose, die door een chemische schakeling verbonden zijn. Om dit voedingssuiker uit de darm op te kunnen nemen is splitsing van lactose in de twee suikers noodzakelijk. Deze splitsing wordt verzorgd door het enzym lactase, dat zich in het slijmvlies van de dunne darm bevindt.
Normaal neemt bij zoogdieren, ook bij de mens, de activiteit van het enzym lactase af na de zoogperiode. Deze leeftijd gebonden afname van de lactase activiteit is erfelijk bepaald. Het blijkt echter, dat er ook mensen zijn die tot op hoog volwassen leeftijd een hoge lactase activiteit behouden en dus als volwassene goed in staat zijn om melksuiker te verteren. Waarschijnlijk komt dit doordat er in het verleden een spontane verandering (mutatie) in het gen dat de lactase activiteit regelt, is opgetreden. In gebieden met weinig zonlicht en relatief weinig calcium en eiwit in de voeding (zoals bij voorbeeld in Noord Europa) was iemand met deze gemuteerde eigenschap waarschijnlijk in het voordeel ten opzichte van mensen met een lage lactase activiteit, omdat ze door melk te drinken meer calcium en eiwit opnamen. Hierdoor hadden ze een hogere levensverwachting, minder kans op botontkalking en kregen zij waarschijnlijk een groter aantal kinderen dan de mensen die geen melk konden verdragen. Omdat ook het behoud van een hoge lactase activiteit erfelijk wordt bepaald, kon deze mutatie worden geselecteerd in deze bevolkingsgroep. De meeste afstammelingen van de oorspronkelijke Noord Europeanen zijn nog steeds in staat om melksuiker te splitsen, terwijl ongeveer 75% van de wereldbevolking (o.a. Afrikanen en Aziaten) dat niet kan.

Naast deze veel voorkomende erfelijke vorm van lage lactase activiteit kan een verminderde splitsingscapaciteit voor melksuiker veroorzaakt worden door een zeer zeldzame aangeboren afwijking, die zich kenmerkt door het optreden van heftige diarree na het introduceren van melkvoeding in de eerste levensdagen. Dit wordt veroorzaakt doordat de lactase activiteit vanaf de geboorte vrijwel volledige afwezig is. De behandeling van deze erfelijke aandoening bestaat uit het levenslang gebruiken van lactosevrije voeding. Ook kan beschadiging van het dunne darm slijmvlies een verminderde lactase activiteit tot gevolg hebben. Aandoeningen die het slijmvlies van de dunne darm kunnen beschadigen zijn o.a. coeliakie (gluten overgevoeligheid), de ziekte van Crohn, behandeling met cytostatica (die de celdeling van het dunne darmslijmvlies remmen) of darminfecties.

Om aan te tonen of verminderde lactose splitsing de oorzaak is van optredende klachten na melkconsumptie zijn in de loop der jaren veel testmethoden ontwikkeld. In *Hoofdstuk 1* worden deze testen besproken en wordt uitgelegd waarom geen van deze testen op voldoende betrouwbare wijze de melksuiker vertering kan meten. De tot op heden meest gebruikte test in de kliniek is de waterstofademtest. Hierbij wordt de waterstof (H_2) concentratie in ademlucht gemeten na inname van lactose. Lactose die niet gesplitst en opgenomen wordt in de dunne darm, bereikt de dikke darm. De daar aanwezige bacteriën verwerken deze suiker, waarbij onder andere H_2 wordt gemaakt. Omdat H_2 nergens anders in het lichaam wordt geproduceerd, is de H_2 concentratie in de adem gerelateerd aan de aanwezigheid van koolhydraat in de dikke darm. Helaas blijken er nogal wat problemen te zijn met deze test: sommige mensen bezitten een bacterieflora die geen H_2 kan produceren of die H_2 onmiddellijk weer consumeert. Medicijn gebruik of factoren die buiten het maagdarmkanaal gelegen zijn, zoals hyperventilatie of roken, kunnen de test verstoren. Ook een lage zuurgraad (pH) in de dikke darm, die ontstaat doordat de bacteriën behalve gassen ook melkzuur kunnen maken uit lactose kan de test verstoren. Hierdoor is er een grote kans op "fout normale" uitslagen (d.w.z. de testuitslag is normaal, terwijl er wel malabsorptie is). Omdat deze test veel gebruikt wordt, hebben we de resultaten ervan vergeleken met die van onze nieuw ontwikkelde test methoden, zoals in de afzonderlijke hoofdstukken wordt beschreven.

In ons onderzoek wordt een alternatieve techniek gebruikt om de lactose splitsing te meten. De basis van deze techniek is gelegen in het feit dat lactose gemerkt kan worden met behulp van een isotoop, waardoor de weg van vertering en stofwisseling ervan door het lichaam kan worden vervolgd. De isotoop die wij gebruikt hebben is ^{13}C. Dit is een koolstofatoom dat iets zwaarder is dan het in veel grotere hoeveelheden in de natuur voorkomende ^{12}C. Door het hogere atoomgewicht is herkenning van dit isotoop door gevoelige meetapparatuur mogelijk (Isotopen Ratio Massa Spectrometrie, IRMS). De gemerkte lactose, ^{13}C-lactose, werd door het Nederlands Instituut voor Zuivel Onderzoek (NIZO)

in Ede geproduceerd. Koeien werden daarvoor gedurende 5 weken alleen gevoed met maïsvoer, een product dat van nature veel ^{13}C bevat. Door deze voeding werd hun melk en daarmee ook de in die melk aanwezige lactose verrijkt met ^{13}C. Vervolgens werd lactose uit de melk geïsoleerd en als poeder aan ons geleverd. Voor onze experimenten werd dit poeder opgelost in water en werd nadat de oplossing door de geteste personen was gedronken in hun uitademingslucht en in bloed gemeten hoe de lactose door het lichaam was verwerkt.

In *Hoofdstuk 2* worden de resultaten van ons onderzoek van de lactose splitsing bij kinderen beschreven. De onderzochte kinderen werden verdacht van een slijmvliesbeschadiging van de dunne darm en moesten in verband daarmee een darm biopsie (het wegnemen van een stukje slijmvlies met behulp van een endoscoop, een kijkinstrument waarmee via de mond, slokdarm en maag de dunne darm kan worden bereikt) ondergaan. Van de verkregen slijmvliesstukjes werd in het laboratorium de lactase activiteit gemeten. De resultaten van deze lactase meting werden vergeleken met die van de ^{13}C-lactose ademtest. De kinderen dronken een ^{13}C-lactose drank, waarna de concentratie van H_2 en $^{13}CO_2$ in de ademlucht werden gemeten vanaf kort voor inname van de drank tot en met 4 uur daarna. De test is gebaseerd op het principe dat ^{13}C-lactose door lactase kan worden gesplitst in ^{13}C-glucose en ^{13}C-galactose. Deze enkelvoudige suikers worden opgenomen door het dunne darm slijmvlies en vervolgens via de poortader naar de lever vervoerd. In de lever wordt ^{13}C-galactose omgezet in ^{13}C-glucose. ^{13}C-glucose wordt daarna in het lichaam gemetaboliseerd waarbij als eindproduct $^{13}CO_2$ (kooldioxide) ontstaat. De hoeveelheid $^{13}CO_2$ die wordt uitgeademd is een maat voor de hoeveelheid ^{13}C-lactose die succesvol is gesplitst, opgenomen en gemetaboliseerd. We vonden dat de $^{13}CO_2$ test de gemeten lactase activiteit beter aangaf dan de H_2 test. De testresultaten waren echter niet zo veel beter dat we het verantwoord vonden om de H_2 test erdoor te vervangen. De enigszins tegenvallende resultaten kunnen mogelijk worden veroorzaakt door het feit dat de $^{13}CO_2$ test in rust wordt verricht, zoals gebruikelijk is voor de H_2 test. Onder deze condities wordt maar een beperkt deel van het opgenomen koolhydraat verbrand tot $^{13}CO_2$, waardoor over het algemeen minder dan 25% van de toegediende testdosis wordt uitgeademd. Verhoging van het metabolisme, bij voorbeeld door meer lichamelijke activiteit tijdens de test, zou dit probleem kunnen verkleinen.

Daarnaast kan uit lactose dat de dikke darm bereikt behalve H_2 ook $^{13}CO_2$ worden gevormd, wat toegevoegd wordt aan het $^{13}CO_2$ dat uit verteerd lactose afkomstig is. Hierdoor wordt de lactase capaciteit op grond van de gemeten testresultaten overschat.

Om de grootte van beide effecten op het testresultaat te bepalen, verrichtten we aanvullend onderzoek.

In *Hoofdstuk 3* worden de resultaten van het verhogen van de lichamelijke

activiteit op het resultaat van de test beschreven.
De resultaten van de $^{13}CO_2$ ademtest van jong volwassen in rust werden vergeleken met die van proefpersonen die gedurende 4 uur op een home trainer met een vermogen van 50 Watt fietsten. Tijdens de inspanningtest bleek, zoals verwacht, dat de totale $^{13}CO_2$ uitscheiding sterk toenam. Eén van de proefpersonen bleek een niet-symptomatische lactose maldigestie te hebben, die werd ontdekt door een afwijkende lactose-H_2 ademtest uitslag. In rust was het resultaat van de $^{13}CO_2$ test van deze persoon niet te onderscheiden van die van de individuen met een normale (hoge) lactose vertering. Bij de inspanningstest nam de $^{13}CO_2$ uitscheiding echter nauwelijks toe en werd het testresultaat dus afwijkend. Tijdens inspanning blijkt de ^{13}C-lactose splitsingscapaciteit de snelheidsbepalende stap van de test te zijn geworden, terwijl dat in rust het ^{13}C-glucose metabolisme was. Hoewel de test onder deze omstandigheden veel betrouwbaarder wordt, zijn de uitvoeringscondities te onpraktisch om op grote schaal klinisch te worden gebruikt.
In *Hoofdstuk 4* wordt het effect van gasvorming in de dikke darm beschreven. In deze studie werd gebruik gemaakt van ^{13}C-lactulose als testsubstraat. Dit is een chemische variant van lactose, die niet kan worden gesplitst in de dunne darm. De volledige hoeveelheid ingenomen lactulose bereikt dus de dikke darm. Omdat de verwerking van lactulose in de dikke darm identiek is aan die van lactose, kan de hoeveelheid uitgeademd gas worden gerelateerd aan de hoeveelheid koolhydraat die door de bacteriën verwerkt is. Bij verschillende personen werden verschillende doseringen lactulose gebruikt om de relatie tussen koolhydraatdosis en H_2 en $^{13}CO_2$ productie vast te stellen. Bij een aantal proefpersonen werden herhaalde testen met een zelfde dosis verricht om de variatie in de gasproductie in de tijd te bepalen. Het bleek dat zowel de vorming van H_2 als van $^{13}CO_2$ erg variabel was en dat er geen duidelijke relatie tussen dosis en respons kon worden aangetoond. Wel bleek een correlatie tussen de H_2 en $^{13}CO_2$ productie te bestaan: bij hoge H_2 productie bleek de $^{13}CO_2$ productie eveneens hoog, bij lage H_2 productie was ook de $^{13}CO_2$ vorming gering. Bij mensen met een geringe gasproductie is de $^{13}CO_2$ test betrouwbaarder dan de H_2 test, omdat de storende invloed van in de dikke darm geproduceerd $^{13}CO_2$ gas in dat geval gering is en omdat door de geringe gasproductie de H_2 test vaak een onterecht normale uitslag zal geven. Bij mensen met een hoge gasproductie is de situatie tegenovergesteld, omdat in dat geval de storende invloed van $^{13}CO_2$ uit de dikke darm groter zal zijn en de H_2 test meer kans heeft om een betrouwbare uitslag te geven. Omdat de gasproductie in de tijd erg bleek te wisselen, is ook de gelijktijdige analyse van beide testen niet geschikt voor praktische toepassing.
Hoofdstuk 5 beschrijft de resultaten van een alternatieve methode om lactose splitsing te meten. Wanneer ^{13}C-lactose door lactase in glucose en galactose wordt gesplitst, zullen beide componenten ook met ^{13}C gelabeld zijn. Hierdoor

is glucose afkomstig uit lactose (^{13}C-glucose) te onderscheiden van glucose die uit in het lichaam opgeslagen glucose voorraden afkomstig is (ongemerkt glucose). De lactose vertering kan worden gemeten door de concentratiestijging van ^{13}C-glucose in het bloed na inname van ^{13}C-lactose te meten. Deze methode werd gebruikt bij 2 groepen vrijwilligers: een groep Chinese proefpersonen met een erfelijk bepaalde lage lactase activiteit en een groep Nederlanders met een, eveneens erfelijk bepaalde, hoge lactase activiteit. Het verschil in lactase activiteit bleek door de test goed te worden aangegeven. Het tijdstip waarop de maximale ^{13}C-glucose concentratie na inname van ^{13}C-lactose optrad, bleek echter nogal te variëren. Dit wordt waarschijnlijk veroorzaakt door individuele verschillen in tempo van maaagontlediging en dunne darm passagetijd. Wij verwachtten dan ook dat de test nog betrouwbaarder gemaakt zou kunnen worden door een aanpassing die in *Hoofdstuk 6* wordt beschreven.

We hebben aan de ^{13}C-lactose oplossing een referentiestof toegevoegd, die op dezelfde wijze als lactose gemetaboliseerd wordt, maar niet tevoren hoeft te worden gesplitst door lactase. De referentiestof was ^{2}H-glucose, eveneens een stabiel isotoop van het normaal in de natuur voorkomende ^{1}H-glucose. Ook deze isotoop kan met behulp van massa spectrometrie worden geïdentificeerd. Door na inname van het mengsel van ^{13}C-lactose en ^{2}H-glucose de verhouding van de concentraties van ^{13}C- en ^{2}H-glucose te meten, kan de lactase activiteit worden bepaald. Immers, bij een lage lactase activiteit zal de ^{13}C-glucose concentratie laag blijven, terwijl de ^{2}H glucose concentratie wel zal stijgen. Bij hoge lactase activiteit zullen beide glucose concentraties stijgen, waardoor de verhouding daarvan (de ratio genoemd) stijgt. Ook deze test werd toegepast in twee groepen proefpersonen met erfelijk bepaalde verschillen in lactase activiteit (Chinezen versus Nederlanders). De testresultaten van beide groepen waren duidelijk van elkaar te onderscheiden met een afkappunt voor de ratio van 0.45. Het beste onderscheid tussen de groep met een hoge en die met een lage lactose verteringscapaciteit bleek te worden verkregen door de gemiddelde waarde van de ratio tussen 45 en 70 minuten na inname van de testoplossing te meten (de Lactose Digestie Index, LDI).

Met behulp van deze nieuwe test hebben we de relatie tussen de mate van lactose vertering en het optreden en de ernst van klachten bij gezonde mensen onderzocht. De resultaten van deze studie worden beschreven in *Hoofdstuk 7*. Na inname van 25 gram lactose (te vergelijken met ongeveer een halve liter melk) door Chinese volwassen vrijwilligers, werd door hen geregistreerd welke klachten optraden. In tegenstelling tot wat wij hadden verwacht, traden bij 35% van de onderzochte mensen helemaal geen verschijnselen op. Bij 40% ontstonden lichte klachten van buikpijn en winderigheid, terwijl bij 25% ook diarree ontstond. De mensen zonder symptomen bleken een hogere LDI en een langere passagetijd van mond tot dikke darm te hebben dan de mensen met symptomen. Bij de personen met milde klachten of die met diarree bleken

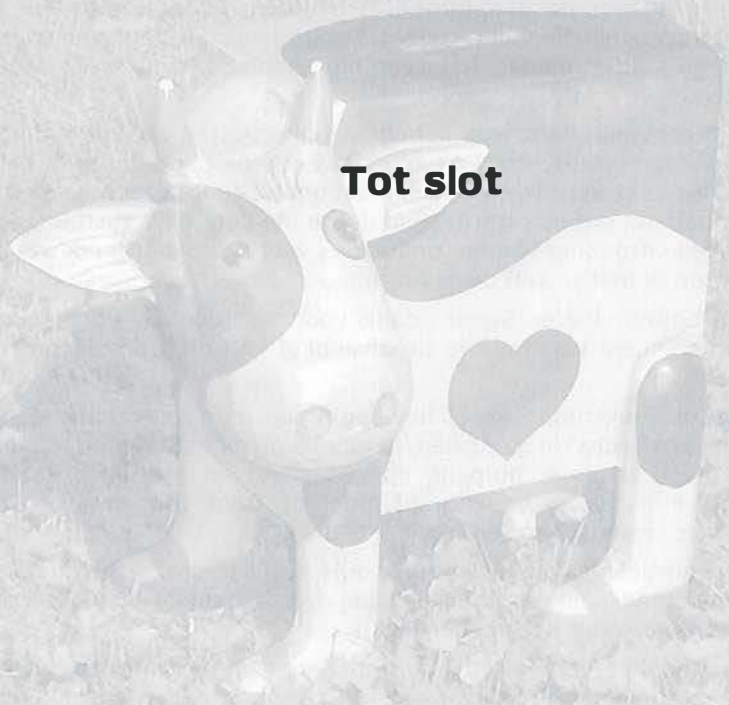
echter geen verschillen van betekenis aantoonbaar in vertering index of passagetijd. Andere factoren dan lactosevertering en passagetijd zijn dus ook van belang bij de aard van de klachten die ontstaan na consumptie van lactose door mensen met een verlaagde lactose verteringscapaciteit. Deze factoren zijn zeer waarschijnlijk gelegen in de aanpassingsmechanismen van de dikke darm. Dit zullen wij binnenkort verder gaan onderzoeken.

Naast de effecten van erfelijk bepaalde verschillen in lactase activiteit, hebben we onderzocht wat de gevolgen van dunne darm slijmvlies beschadiging zijn op het lactose splitsende vermogen. De resultaten van dit onderzoek staan beschreven in *Hoofdstuk 8*.

Bij kinderen die een dunne darm biopsie moesten ondergaan wegens verdenking op slijmvlies beschadiging onderzochten we de lactose splitsing met behulp van de $^{13}C/^{2}H$ test. We vonden dat bij kinderen zonder slijmvliesbeschadiging de lactose verteringscapaciteit meestal normaal was, maar dat bij kinderen met slijmvlies beschadiging de gemeten lactase activiteit in het biopt meestal lager was dan de met de test gemeten activiteit. Dit kan worden verklaard door het feit dat slijmvliesbeschadiging meestal niet homogeen is, maar dat sommige stukken erger zijn aangetast dan andere, waardoor het extrapoleren van de gemeten activiteit van het biopt naar de werkelijke activiteit eigenlijk niet verantwoord is.

Wij concluderen in *Hoofdstuk 9* op grond van de beschreven studieresultaten dat de nieuwe $^{13}C/^{2}H$-glucose test de lactose vertering betrouwbaar kan meten en dus veel mogelijkheden biedt voor diagnostiek. Dit geldt zowel voor klinische vraagstellingen alsook voor het bestuderen van de erfelijke variaties in lactase activiteit en het beoordelen van factoren die van invloed zijn op het al dan niet optreden van symptomen bij mensen met een verminderde splitsingscapaciteit voor lactose.

Door gebruik te maken van deze meetmethode zal meer inzicht kunnen worden verkregen in fysiologie en pathofysiologie van lactose vertering bij mensen, waardoor een goede behandeling van mensen met een lage lactase activiteit beschikbaar kan komen. Daarnaast kunnen methoden om de lactose vertering te verbeteren en het optreden van ongewenste symptomen te beperken geëvalueerd worden. Ten slotte zal het mogelijk worden om de relatie tussen genetische achtergrond (genotype) en het effect daarvan op de lactase expressie in de darm (fenotype) op te helderen.



Tot slot

Tot slot:

Het is af! Na een lange periode waarin klinische training, wetenschappelijk onderzoek en onvoorziene problemen zich afwisselden, maar ook vaak tegelijkertijd optraden, ligt hier het resultaat. Dank zij hulp en steun van vrienden, familie en collega's is het gelukt. Iedereen die geholpen heeft wil ik daarvoor hartelijk danken.

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Levensloop:

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Getrouwd met Tonny van Gemert